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The Official Periodical of
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Organe Officiel de
L'ACADEMIE INTERNATIONALE DE CYTOLOGIE GYNECOLOGIQUE

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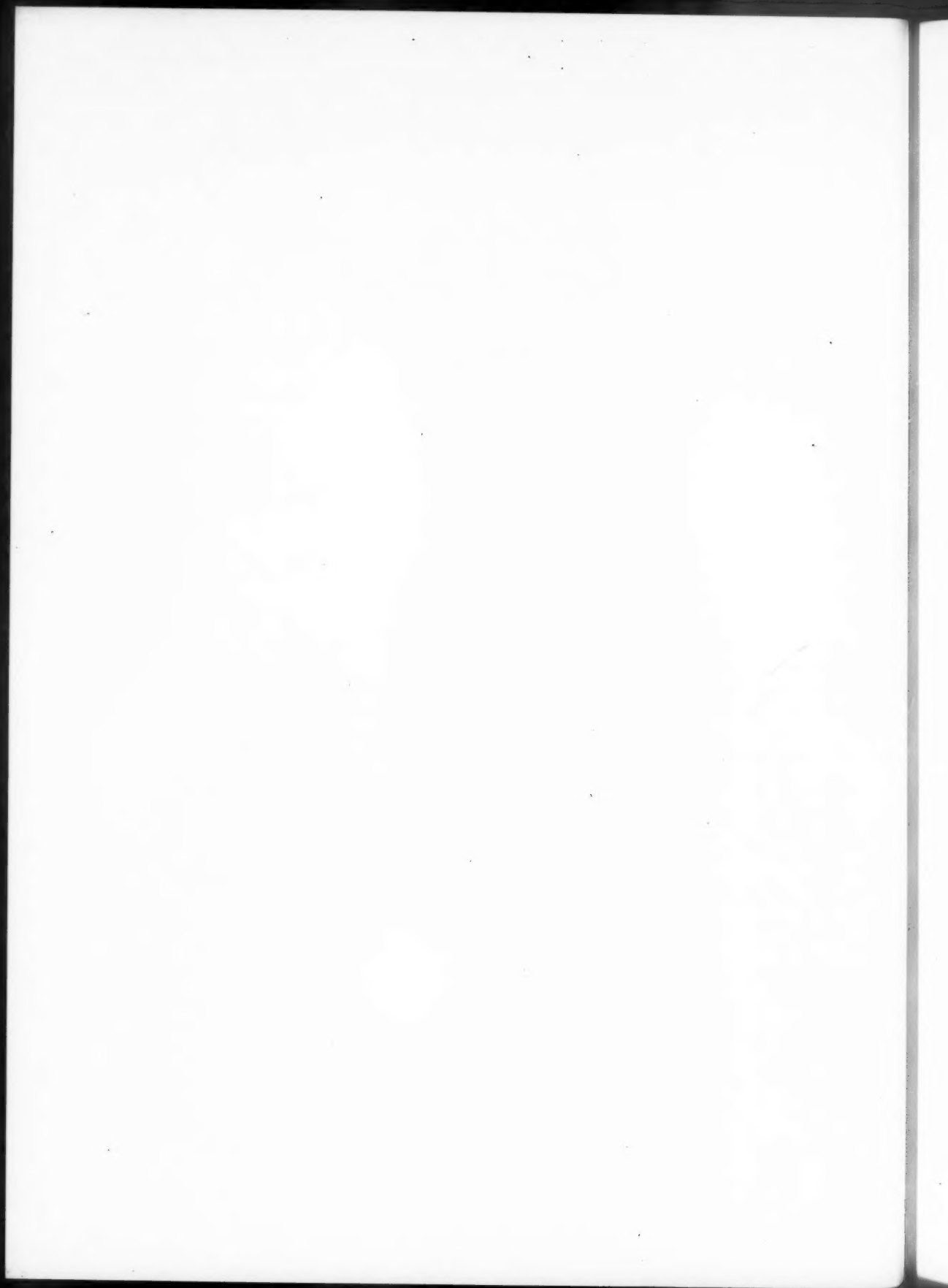


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LETTERS TO THE EDITOR

COLPOSCOPICAL AND CYTOLOGICAL ALTERATIONS DURING PREGNANCY

TO THE EDITORS:

Reference is made to the discussions on colposcopy and cytology during pregnancy in the Symposium by Correspondence of Vol. III, No. 1, 1959 of ACTA CYTOLOGICA.

We completely agree with de Brux that the differential diagnosis - corresponding to the histopathology of the modifications of the epithelium of the cervix - during pregnancy is most difficult. The different hormonal influences, the increasing inflammatory appearances (increase of the leukorrhea), surely lead, if they appear in the smear of non-pregnant women, to a suspicious and even to a positive finding. Therefore, we are very prudent in the judgment of smears with doubtful increasing atypical epithelium or questionable carcinomatous alterations during pregnancy. We do not wish to discuss the usual "normal" pregnancy smear. As the studies of Ober, Hamperl and Kaufmann show, the critical zone during pregnancy (contact of the columnar epithelium with the squamous epithelium) is displaced to the external surface of the cervix.

As the colposcopic pictures will show, at certain places on the surface of the cervix, an extraordinary hyperemia is seen, also quite often growing formations of epithelium, which histologically can be atypia of a low degree, as well as a questionable carcinoma.

A. In the smears great variations of cells are seen which we classify (according to the Papanicolaou classification) as follows:

1. Typical navicular cells without nuclear anomalies correspond to Class I.
2. Should we find in addition to the navicular cells, single, cyanophilic parabasal cells with irregular nuclei, low hyperchromasia, inflammatory appearances, as well as large, irregular, naked nuclei (which are even larger than histiocytes and are designated by de Brux as decidua cells), we classify the smear as Class II. Repetition of the smears at short intervals is always necessary. The result is that one usually finds histologically abnormal or simple atypical epithelium (Hinselmann I/II a-c) and sometimes inflammatory modifications.
3. It is more difficult when so-called "dyskaryotic cells" appear. Here are irregular polychromatic nuclei in which the chromatin structure is still recognizable. The nuclei usually lie in an eosinophilic cytoplasm and can be better visualized. The cytoplasm is not necessarily eosinophilic and may be stained cyanophilic. Smears with such cellular elements are always classified - if taken from either a pregnant or non-pregnant woman - as suspicious in Class III.

Here we agree with Nieburgs that the criteria for suspicious, i.e., positive smears, are always the same, whether during pregnancy or not.

A definitive treatment based on such smears should never be executed. The appearance of dyskaryotic cells during pregnancy only indicates further exploration, i.e., repeated smears and perhaps biopsies under the direction of colposcopy with cauterization following.

4. We speak of a positive smear during pregnancy when all the criteria of marked atypia appear, namely anisokaryosis, anisocytosis, hyper- and polychromasia, as well as cells of different degrees of maturity with polymorphic alterations. Whether or not these cells come from a superficial or a grave modification or even from an invasive carcinoma, cannot be judged from the smear alone. We wish to point out that a differential diagnosis "carcinoma in situ" or "invasive carcinoma" is not possible cytologically. This is still more difficult in the pregnancy smear and may be danger-

ous, since often cases of the smallest carcinomatous changes are seen which are not observed post partum or post abortum. Simply to begin therapy as a result of a positive smear would carry with it the danger of an unfounded, mutilating operation. Repeat directed biopsies, plus repeat smears, are specially indicated here; in other words, preferably a conservative position should be taken.

B. The colposcopic changes during pregnancy are easily recognized from one point of view, but from another point of view they are difficult to interpret.

1. If new modifications appear, then we are dealing with ectopies, which may secondarily regenerate. In pregnancy these ectopies are especially characteristic, coarsely papillary and sharply divided from the squamous epithelium. If a regeneration occurs during pregnancy - then as a result of the increased hydration, hyperemia and hormonal and inflammatory influences - an atypia may easily occur. Whether or not we are dealing with newly formed epithelial changes may only be determined when the patient has been observed before or at the beginning of pregnancy.
2. Physiological modifications (ectopy, zone of transition, localized and generalized vaginitis) which already occurred before pregnancy exacerbate easily and often take on an atypical appearance. Irregular proliferations are especially noted at the border between the columnar and squamous epithelium. If such tissue is easily vulnerable and bleeds easily, a biopsy should always be performed.
3. As a result of the hyperemia and increased hydration we find, as does Bret, increasingly the appearance of the colposcopically atypical epithelium (as is seen with dabbing with 2% acetic acid). In this way changes such as "ground," "mosaic" and "leukoplakia" may easily be seen. This colposcopically atypical epithelium should, even in pregnancy, be taken seriously, even when histologically only a simple epithelial atypia and a low grade of "unrest" are found. The colposcopically most difficult, most characteristic and most atypical alterations remain in the so-called "atypical zone of transformation" which exhibits a high percentage of carcinomatous changes. This is true for pregnancy and non-pregnancy. The irregularity of the tissue, easily vulnerable and bleeding points, chaotic vessel courses, which transparently look like "ground," are characteristic for this modification. With the Schiller test these sections stain yellowish-white and also show iodine positive spots, the vessels remaining red and bleeding easily. A differentiation from the coarse papillary ectopy is easily possible (the latter always shows the grape-like modifications of the mucous membrane). One or more biopsies must be executed during pregnancy at the site of such atypical regeneration.

In summary, one may say, in agreement with most authors (Bret, de Brux, Nieburgs, and others), that colposcopy and cytology are precious aids in judging epithelial modifications of the cervix during pregnancy. Both methods should always be used.

Bibliography

1. Bret, J.: Acta, Unio Internat. Contra Cancrum 4, 1958.
2. Bret, J. and Coupez, F.J.: ACTA CYTOLOGICA 3:No. 1, 61, 1959.
3. de Brux, J.: Acta, Unio Internat. Contra Cancrum 4, 1958.
4. de Brux, J.: ACTA CYTOLOGICA 3:No. 1, 44, 91, 1959.
5. Kaufmann, C., Ober, K.G. and Hamperl, H.: Arch. f. Gynaek. 184:181, 1954.
6. Nieburgs, H.: ACTA CYTOLOGICA 3:No. 1, 77, 91, 1959.

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CERVICAL CARCINOMA DURING PREGNANCY

TO THE EDITORS:

Reference is made to the papers by Drs. Emmerich von Haam, (Exfoliative Cytology of Invasive Carcinoma During Pregnancy) and Friedrich Bajardi, Jules-André Bret and F.J. Coupez (Diagnostic Accuracy of Colposcopy as Compared with Cytology in the Detection of Cervical Carcinoma During Pregnancy) which appeared in ACTA CYTOLOGICA, Volume III, No. 1, 1959, (pp. 98, 107).

Cervical scrapings during pregnancy are still seldom performed. It is by no means a routine procedure. Our laboratory receives smears from about 2300 patients a year for cancer detection. Only about 5% are pregnancy smears. Our experience in this limited collection of cases allows the following statements:

1. Cervical scraping smears obtained from pregnant women make possible the detection of carcinoma in situ and preclinical invasive carcinoma of the cervix.

2. More skill and endurance, i. e., more time, is required to take the smears because the speculum examination of the cervix during pregnancy is more troublesome (discharge, bleeding).

3. The interpretation of pregnancy smears is not essentially different from those of non-pregnant patients. (Figures 1a, 1b, 2a, 2b)



low power



high power

Fig. 1a. Carcinoma in situ involving cervical glands, 13th week of pregnancy.

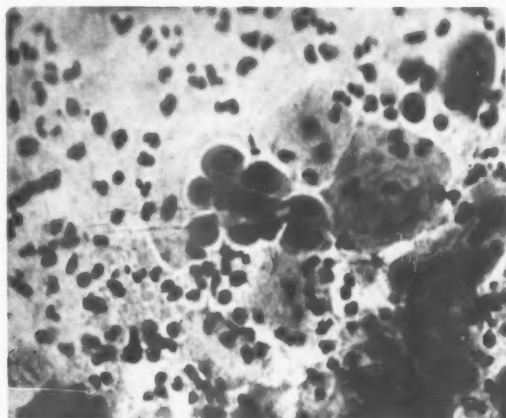
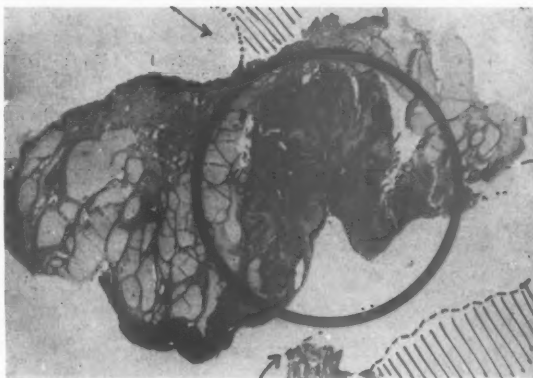


Fig. 1b. The corresponding smear with a cluster of malignant cells.

Fig. 2a. Early invasive carcinoma of the cervix. Low magnification of a section through the whole posterior lip of the cervix. Note the intra-cervical localization of the small tumor. Clinical examinations of the cervix by the naked eye and with the colposcope were negative. (The tumor was not detectable clinically by visual examination and/or colposcopy.)



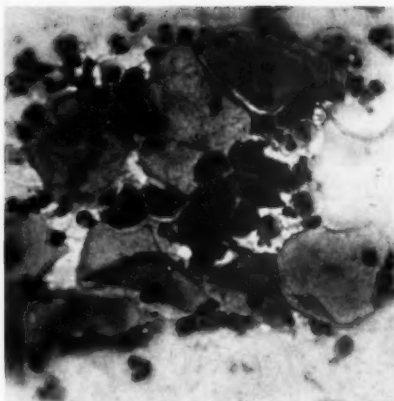


Fig. 2b. The corresponding smear showing numerous malignant cells amid superficial and intermediate cells.

4. There are no cytological "pregnancy-changes" simulating or mimicing pre-invasive or pre-clinical invasive carcinoma of the cervix.

5. We are of the opinion, in agreement with Greene and co-workers and Marsh and co-workers, that lesions such as carcinoma in situ or very early invasive carcinoma persist after termination of the pregnancy. One case with a positive smear in the 20th week of pregnancy, confirmed by biopsy as carcinoma in situ (v. Albertini), was controlled periodically by smears. Three years later a control biopsy revealed an early invasive carcinoma (v. Albertini). (Figs. 3a, 3b, 3c.)

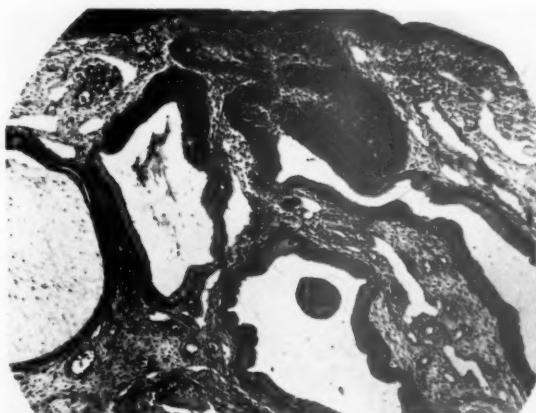


Fig. 3a. Carcinoma in situ partly filling the endocervical glands, 20th week of pregnancy.

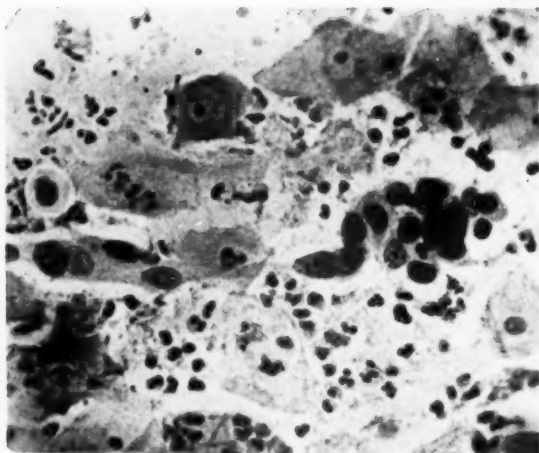


Fig. 3b. The corresponding smear with numerous malignant cells.

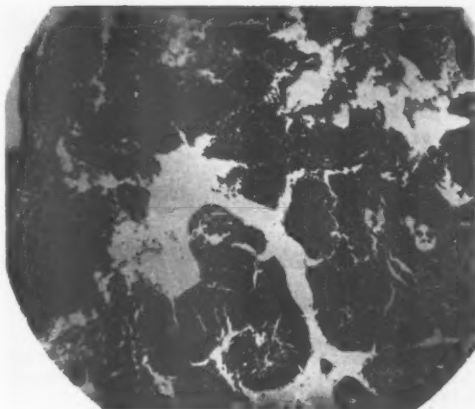


Fig. 3c. The same case 3 years later. A very early invasive carcinoma (diameter 3 mm) was detected on the external os.

6. The high rate of 2.5% of carcinoma in situ and/or carcinoma with minimal invasion which was found by Kaufmann and co-workers, by routine 12 o'clock biopsies in an unselected collection of 360 pregnant patients, seems not to be reflected in our pregnancy smears. The number is still too small to allow definitive conclusions.

Bibliography

1. Greene, R.R. and Peckham, M.B.: Am. J. Obst. and Gynec. 75:551, 1958.
2. Marsh, M. and Fitzgerald, P.J.: Cancer 9:1195, 1956.
3. Hamperl, H., Kaufmann, C. and Ober, G.K.: Arch. f. Gyn. 184:181, 1954.

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MANAGEMENT OF CARCINOMA IN SITU AND INVASIVE CARCINOMA DURING PREGNANCY

TO THE EDITORS:

Colposcopy performed regularly during the first half of pregnancy revealed the wide variety of mucosal pictures of the uterine cervix during pregnancy, as has been known for a long time. This phenomenon is not only caused by the succulence and cyanosis of the tissues of the frequently occurring ectopies and transformation zones, but also by new formation and hyperemia of the vessels. More recent cytological investigations on routine vaginal smears and biopsies taken from the anterior lip of the external os suggest an increased incidence of carcinoma in situ during pregnancy.

It is not yet quite clear if we are dealing with a definite precancerous state or with a temporary atypical epithelial proliferation due to the unique requirements of pregnancy, which are frequently combined with secondary inflammatory reactions.

Frequently repeated colposcopic and cytological control examinations often suffice for clarification, and biopsy is not necessary. Only when the colposcopic pictures are suspicious and cytological findings remain positive, especially when there is a history of bleeding, does histological verification of the diagnosis have to be requested. The biopsy must not be too extensive because the pregnancy may be endangered by the procedure and the concomitant bleeding. However, if the material is too scant for histological evaluation, the pre-therapeutic histological diagnosis becomes uncertain and it can not be decided in a given case whether a pre-invasive or very early invasive carcinoma (microcarcinoma) is being dealt with.

Only when the invasive and destructive growth is confirmed with certainty is a radical treatment indicated during the first months of pregnancy. During the second half of pregnancy local application of radium may be applied in cases of very early carcinoma in young women when desire for children is present, without fear of endangering the child. Cesarean section combined with the radical operation of Wertheim is done at term. From our own experience we can reconfirm this recommendation of Bickenbach.

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VAGINAL FLORA DURING PREGNANCY

TO THE EDITORS:

Reference is made to the paper "Vaginal Flora of the Pregnant Woman" in *Acta Cytologica*, Volume III, No. 2, page 222, 1959.

I have described the different forms of cytolysis encountered and their microbiological aspects as studied with the phase contrast microscope. The one caused by a short bacillus is possibly due to a peculiar form of Döderlein bacillus. Bacteriological studies could be very easily made on this subject since this germ, if not the only one is at least predominant.

The two other types of cytolysis, the Döderlein type and the micrococcus type are not only more marked but also more frequent during pregnancy. I am at the present working on a statistical study on this subject. I believe the Pseudo-mycelous Döderlein is not frequent during pregnancy.

To my knowledge nobody has examined the frequency of association of leptothrix with *Trichomonas* in the third month of pregnancy, up to now. In the majority of the cases one can find *Trichomonas* when leptothrix is present. It seems we deal here with a true symbiosis, although the elimination of leptothrix by local therapy does not always eliminate completely the trichomoniasis.

Concerning *Trichomonas* one should recall the findings of Van Meensel who also used the phase contrast microscope. At the end of pregnancy, he could usually observe signs of damage of the *Trichomonas* slowing down of the undulant membranes and of the flagellar movement. The trichomonads become shaped progressively rounder and immobile and eventually take the aspect of a round pseudo-cyst with a bright peripheral cuticula. We believe indeed that we are faced here with a true encysted form, proper to the end of pregnancy which is apt to resist the stress conditions during delivery.

The non-parasitic, non-specific vaginitis during pregnancy is characterized by the presence of numerous isolated and grouped leukocytes and abundant bacterial flora of great variety. This situation can even be encountered at the eve of delivery. It is possible that this may contribute to the inflammatory complications of the puerperium and post-partum period.

The presence of histiocytes during pregnancy is rare in the vaginal secretions. They are easily recognized by phase contrast microscopy owing to their yellowish appearance, their rounded or oval-shaped form, their peculiar nucleus and their foamy cytoplasm. Histiocytes may exceptionally be encountered in cases of threatened abortion and their occurrence seem to indicate poor prognosis in these cases.

Bibliography

1. Van Meensel, F.: Gestation et Trichomonase Vaginale. *Bull. Soc. Royale Belge de Gynec. et Obstetr.*, 27:227, 1957.

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THE MARKEDLY ATYPICAL CERVICAL EPITHELIUM DURING PREGNANCY AND ITS ALTERATIONS IN THE POSTPARTUM PERIOD

TO THE EDITORS:

Reference is made to the discussions in the Symposium by Correspondence on Cervical Carcinoma During Pregnancy of Vol. III, No. 2, 1959 of *ACTA CYTOLOGICA*.

Many authors refuse the diagnoses "markedly atypical epithelium" or the "so-called intraepithelial carcinoma," which are diagnosed with cervical biopsies during pregnancy (Novak, Te Linde). They refer to the occasionally seen epithelial proliferations during pregnancy, which morphologically exhibit the criteria of malignant alterations of squamous epithelium, but which after pregnancy--without any therapy--could not be found. It is believed that the hormonal impulses during pregnancy lead to a specific proliferation of the squamous epithelium of the cervix which makes it become a so-called "intraepithelial carcinoma," without reaching its prospective malignancy.

Epperson, Deelmann, Galvin and Busby found five cases of so-called "intraepithelial carcinoma" among 286 pregnant women between the ages of 16 and 40, in biopsies taken at different times during the pregnancy, which were not present after the delivery. Similar observations were mentioned by Nesbitt, Pots, Hellmann, and others.

Greene, Peckmann and co-workers found in 14 patients during pregnancy a so-called "carcinoma in situ" which could not be seen in the operative specimen in two cases, seven and eleven weeks after delivery. In the other 12 cases which were operated on four to 29 months after delivery, the "carcinoma in situ" was still present.

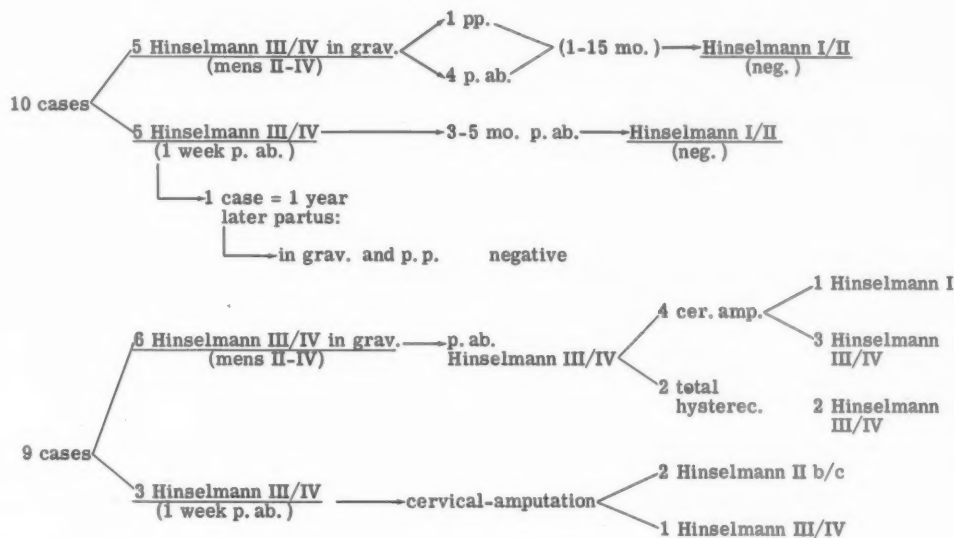
Several cases were reported in which a "carcinoma in situ" was diagnosed during pregnancy and later turned into an invasive carcinoma.

Garson and Gall mentioned a 32 year old pregnant woman in her fourth month of gestation who had a total hysterectomy because of a "carcinoma in situ." The patient died two years later of a carcinoma of the vaginal wall.

Taylor diagnosed, by means of several biopsies, a superficial carcinoma in a patient in the fourth month of pregnancy that became invasive after delivery. Limburg described a similar case with amputation of the cervix post partum. The histological specimen showed an invasive carcinoma. These last cases prove that the increased atypical epithelium of the cervix during pregnancy cannot be considered as being different from those seen in non-pregnant women.

Among our 250 early cases we have 19 women in their second to fourth month of pregnancy who have had an "increased atypical epithelium" during pregnancy (11) or one week post abortion or after an interruption (eight cases). All our patients were in the first half of pregnancy. The youngest patient was 19, the oldest 48 years old (average 30.9 years).

These 19 cases present the following results:



In 11 of our patients having no symptoms we performed, during pregnancy, a prophylactic colposcycological smear examination.

Five of these did not show, one to fifteen months after completion of pregnancy, any "marked atypia" but only a "simple atypical epithelium" (Hinselmann I/II bc). In two of these women a vaginal interruption after psychiatric indication was performed in the second month. One patient had a spontaneous abortion and another one a missed abortion. Only one of these patients came to term. No suspicious epithelium modification could be found (two controls) three to five months after delivery.

In six other cases with suspicious histology during pregnancy (Hinselmann III/IV a-c) the biopsies taken one to four months after delivery were still suspicious. Four times an amputation of the cervix and twice a total hysterectomy (one to four months after an abortion) was performed. Only once did the histology of the cervix show a simple atypical epithelium. In the other five cases the primary suspicious findings were seen again in the operative specimens.

In another group of eight patients of whom six entered the clinic because of a completed abortion and two for a tubal pregnancy, systematic colposcycology could be done eight days after the end of pregnancy. At this time a markedly atypical epithelium could be found in all patients in the cervical biopsies. Further examinations after two, four, and five months revealed negative results in five cases. One of these women had another pregnancy in the same year. During the pregnancy and after delivery no suspicious epithelial modification of the cervix could be found.

In three patients a cervical amputation was performed, one, two and five months after the pregnancy because in repeated examinations markedly atypical epithelium was diagnosed. In the operative specimen only twice was a simple atypical epithelium to be seen and in one case the markedly atypical epithelium was confirmed.

In the 19 pregnant women with markedly atypical epithelium the repeated biopsies were negative ten times altogether (one to fifteen months after the delivery or abortion). Other authors (Treite, Limburg, and others) have the same opinion, that in such cases suspicious parts are taken away by the biopsy or that spontaneous healings occur.

Two cases are especially interesting. In the first case the atypia could no longer be found after an abortion. The second case was a woman with an abortion and a shortly following new pregnancy. During and after this second pregnancy the "suspicious atypical epithelium," which was found originally, could no longer be seen.

In nine cases the "markedly atypical epithelial alterations" remained present after the end of pregnancy. All of these patients were operated on (seven cervical amputations and two total hysterectomies). In six cases the increased atypia could be found in the operative specimen. It is striking that according to the histology, in all the 19 cases, colposcopic and cytological suspicious or positive findings could be confirmed.

The question of whether or not the frequently appearing marked epithelial atypias in the pregnant cervix are true carcinomatous alterations or if they are reversible modifications is still to be discussed. The question of which alterations are already malignant and invasive and which are reversible has to be clarified. Pots compiled the results of many examinations of this question which were published 30 years ago by Stiewe and Adler, and later on by Epperson, Nesbitt and Hellmann, Danforth, Glass, Rosenthal, Murphy and others. According to these authors the following histological changes in the pregnant cervix appear more frequently: glandular hypoplasia, epidermization, thickening of the squamous epithelium, hyperactivity of the basal cells in the connective tissue, edema and inflammatory infiltrations. According to these authors in 0.1-0.6% of the cases a so-called "intraepithelial carcinoma" was observed.

These examinations confirm the well-known increased edema formation and tendency toward inflammatory processes during pregnancy, which can be considered as a result of the marked ectopy of the columnar epithelium of the cervix. The hormonal changes during the pregnancy possibly help to form the thickening of the squamous epithelium. Out of all these examinations no connections to the morphologically new picture of the so-called "intraepithelial carcinoma" could be deduced.

Recent studies of Kaufmann, Ober and Hamperl (1954), similar to the examinations of Pund and Greene, are of great importance. In 500 pregnant women a cervical biopsy was taken at 12 o'clock. The average age of these patients was 29 years. In a high percentage (3%) of cases these authors could diagnose a so-called "intraepithelial carcinoma." Focken and Franz had similar results. In 702 biopsies of the pregnant cervix there were 3% so-called "carcinoma in situ."

Kaufmann and co-workers compared the papers of Clemmesen and Nielson concerning the cervical carcinoma morbidity in Denmark (0.033% to the age of 34 years). The result of their examinations was that the epithelial alterations during pregnancy diagnosed by them were not real carcinomas, nor the real beginning of carcinomas.

According to these papers we notice in our 19 cases with increased atypical epithelium during pregnancy, the frequently seen involution of the suspicious modifications after pregnancy. Ten times a normal colposcopic and cytologic state of the cervix or just a minor alteration (Hinselmann I/II) could be diagnosed a few months after pregnancy. In three patients the marked atypia could not be demonstrated in the operative specimen.

According to the papers of Ober and Gusberg a retraction of the atypia in the center of the cervix can be expected post partum and post abortum. The colposcopic finding as well as the biopsy should become negative. It is interesting, however, that in our cases the cytological results (cervical smears) in the 13 negative cases did not show any more atypical cell elements, which corresponds to the histological diagnosis. Six times no suspicious material could be obtained from the cervix, not even by fractionate curettage.

We share the opinion of Kaufmann, Ober, Hamperl, Pund and Greene that the point of predilection for the "markedly atypical epithelium" is at the anterior lip of the external cervical os. In 16 out of 19 cases the suspicious point was found between ten and two o'clock. These findings agree with Vöge's examinations, who demonstrated that the blood vessel supply is poorest in the midline of the cervix. The more frequent appearance of these matrix areas at 12 o'clock can be explained by Nothdurft's theory, which says that ischemic tissue poorly supplied with blood favors the growth of a tumor.

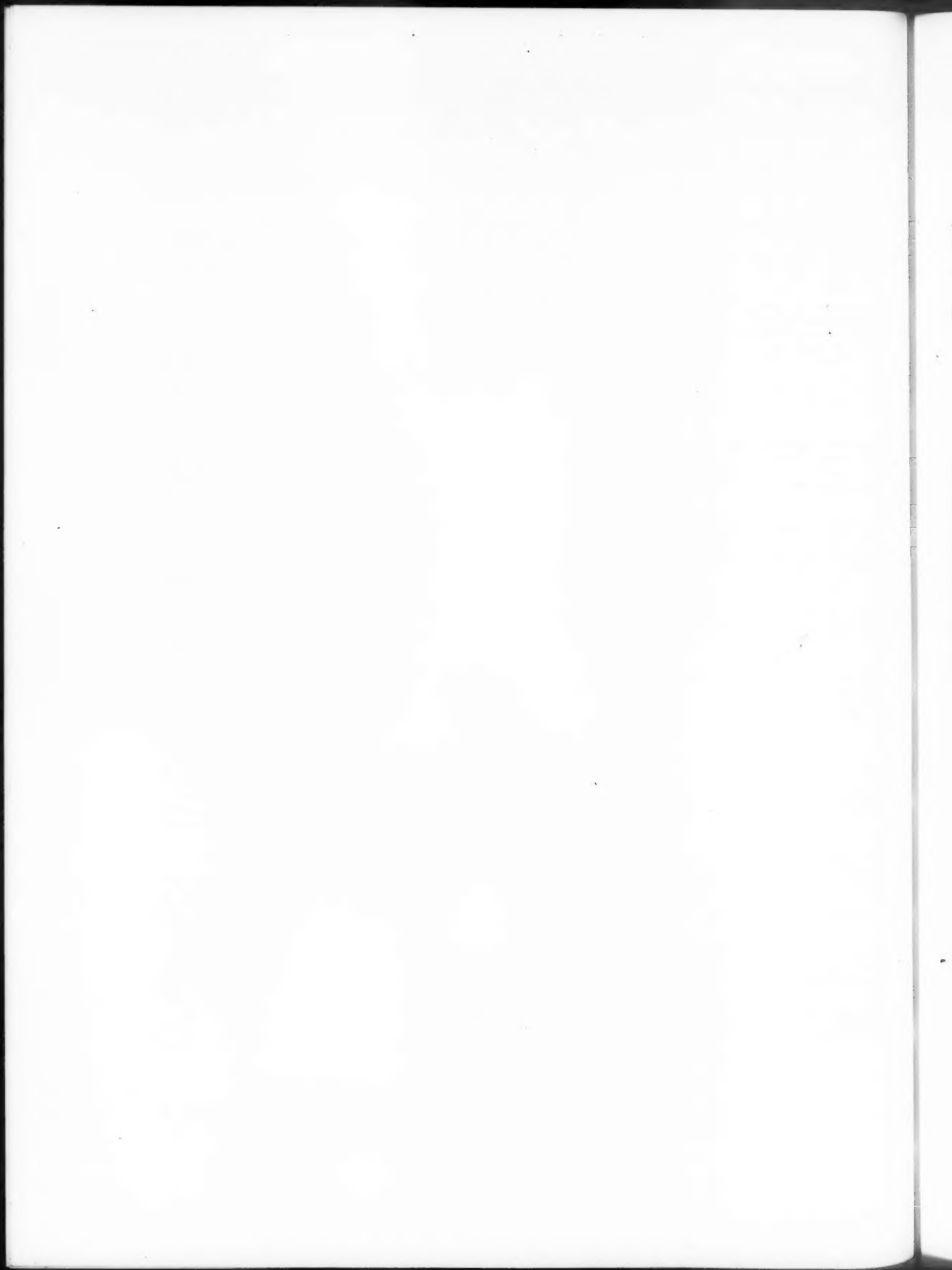
To outline a procedure for handling the markedly atypical epithelium during pregnancy would be a risk with the small number of our cases. We can accept the fact that the origin of marked atypia in the pregnant cervix is favored by hormonal, inflammatory and edema forming influences and by disturbances of the blood supply. These changes can persist during several pregnancies without involution. This point of view is supported by the fact that over 85% of our patients with suspicious and positive findings had one or more pregnancies. The greater number belong to the multipara. Therefore, the conclusion is possible that for markedly atypical epithelium alterations during and outside of pregnancy, the same standard has to be applied. Only the treatment is different depending on the individual case.

Bibliography

1. Adler, K.: Arch. f. Gynaek. 134:504, 1928.
2. Clemmesen, J.: Acta Unio Internat. Contra Cancrum 7:140, 1952.

3. Danforth,: Amer. J. Obst. and Gyn. 60:985, 1950.
4. Deelmann, H.T.: Zbl. f. Krebsforsch. 38:648, 1933.
5. Epperson, J.W. and co-workers: Amer. J. Obst. and Gyn. 61:1, 1951.
6. Focken, A. and Franz, G.: Gebh. u. Frauenh. 9:790, 1956.
7. Galvin, G.H. and Te Linde, R.W.: Amer. J. Obst. and Gyn. 57:15, 1949.
8. Greene, R.: Surg. Obst. and Gyn. 96:71, 1953.
9. Gusberg, S.B.: Amer. J. Obst. and Gyn. 65:1073, 1953.
10. Kaufmann, C., Ober, K.G. and Hamperl, H.: Arch. f. Gynaek. 184:181, 1954.
11. Limburg, H.: Die Frühdiagnose des Uterus-Karzinoms. Stuttgart, 1956, Georg Thieme.
12. Murphy, E.J.: Amer. J. Obst. and Gyn. 59:384, 1950.
13. Nesbitt and Hellmann: Surg. Obst. and Gyn. 94:10, 1952.
14. Nothdurft, H.: Zbl. f. Krebsforsch. 56:176, 1948.
15. Novak, E.R. and Galvin, G.H.: Amer. J. Obst. and Gyn. 62:1079, 1952.
16. Pots, P.: Zbl. f. Gynaek. 76:1041, 1954.
17. Pund, E.: J.A.M.A. 131:960, 1946.
18. Taylor, H.C.: Amer. J. Obst. and Gyn. 52:451, 1946.
Amer. J. Obst. and Gyn. 69:3, 1955.
19. Treite, P.: Zbl. f. Gynaek. 1941: 22; 1941: 1096; 1942: 1458; 1942: 1570.
20. Vöge,: Geburtsh. u. Frauenh. 627:9, 1949.

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The Epithelia of the Uterine Endocervix

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INTRODUCTION

The gynecologist is familiar with the histological pattern of the endocervix with its single-layered cylindrical epithelium which lines the glandular tubules, as well as the endocervical surface epithelium. Abundant mucus productions, which can be demonstrated with the Periodic-Acid-Schiff reaction, obscures to a great extent the fine structure of the cytoplasm. This may be the reason why the ciliated cells which occur in the histological sections in small numbers adjacent to the mucus-producing cells cannot be easily discerned. The existence of ciliated epithelia in the uterine endocervix has been known for quite some time. These cellular elements were described as early as 1908 by Hoehne (3) and 1911 by Mandl (4) and were extensively discussed by Stieve in 1927 (10) in his work on the uterine cervix which was illustrated by drawings made from histological sections. Some authors question the existence of such cells in the endocervix under normal physiological conditions (5), while others assert that all the epithelial cells in the endocervix are ciliated (12).

The histological patterns of the endocervix, with special reference to the changes occurring during the menstrual cycle, were recently dealt with by Topkins (11) and Bradburn and Webb (1). The latter found individual cells with kinocilia in 42% of the cervixes studied.

Since exfoliative cytology has come into general use, the mucus producing cells and ciliated cells of the endocervix are both common knowledge as they are frequently encountered in cytological smears. These elements are excellently depicted in color in the monograph of Papanicolaou, Traut and Marchetti (6) and also in the *Atlas of Exfoliative Cytology* by Papanicolaou (7).

PURPOSE OF PRESENT PAPER

It is the purpose of the present paper to describe the fine structure and particularly the vital structure of the ciliated endocervical cells and to investigate the distribution of the epithelial cells in the endocervix as related to age and menstrual cycle.

MATERIAL AND METHOD

For the study of the vital structure of the endocervical cells, smears have been prepared from fresh surgical specimens without any macroscopical signs of pathology. The specimens

were prepared with a small wooden spatula or a small knife and the material smeared on glass slides and suspended in tyrode solution, avoiding any squeezing of the cells. The specimens were studied immediately under the phase contrast microscope or anoptal microscope. To obtain greater resolution in higher microscopical magnifications the anoptal equipment was preferred for this study. Further technical details pertaining to the preparation of the specimens and information on the anoptal equipment were described elsewhere (8).

Several cytological smears were prepared from each of the 200 cervixes studied. Microscopical scanning has been done under low power. Conspicuous details were photographed under oil immersion. The microphotographs were usually taken not more than 15 minutes after preparation of the slide and not more than 25 minutes after extirpation of the uterus, in order to exclude postmortal changes.

In order to be able to prepare a microphotograph of these cells, it was necessary to cool the slide (e.g., by putting a little ice cube on top). After warming to room temperature, the movement of the cilia began again. Another way to stop the motion was to add some drops of 30% alcohol to the tyrode solution. This low concentration of alcohol did not cause any appreciable morphological changes but stopped the ciliary motion immediately.

We used a Zetopan microscope manufactured by Reichert of Vienna, with anoptal objectives and with the Polyphos condensor. The light source was a circonium burner. In each case the history of the patient, the age, the menstrual phase and other relevant data of the patient were registered.

In other smears the endocervical cells were also stained according to the Papanicolaou technique and were studied as routine cytological material. In each case the relevant data of the patient have been known to the cytologist. In individual cases the same smear which was studied under the anoptal microscope has been fixed and stained following study of the vital structure.

In order to study the type of movement of the kinocilia, a film camera was attached to the microscope (Bolex H 16 M) and motion pictures were taken. A mercury high pressure lamp was used as a light source for the motion pictures.

Numerous histological sections were examined in order to observe the cells in their tissue context. For more than two years notes have been taken on over 1000 histological sections of the uterine endocervix concerning age and phase of the menstrual cycle. Sections of 4 μ thickness have been stained with hematoxylin (Mayer) and eosin. Fixation was done in formalin. A few sections have been fixed according to Bouin and stained according to Goldner. In 200 patients of various

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ages the site of the internal os has also been studied. Care has been taken in preparing these latter sections in order to show endometrium on one side and endocervical tissue on the other side of the histological specimen. The treatment of these sections was the same as above.

RESULTS

The vital structure of the mucus-producing cells of the uterine endocervix varies according to the stage of secretion. High cylindrical cytoplasm (Fig. 1) is displayed by the cells in the early stage of mucus production. The oval-shaped nucleus lies in the longitudinal axis and in most cases shows two nucleoli. The free-cell membrane is well defined and is either straight or slightly convex. During this early stage of mucus production we find within the cells only a few mucus droplets with marked light reflections and, in all cases, some filament-like formations, the significance of which is not quite understood. In smears stained according to the Papanicolaou technique these same cells appear clearly cyanophilic.

Increased mucus production is expressed by an increase in the number of mucus droplets in the cytoplasm (Fig. 2). The

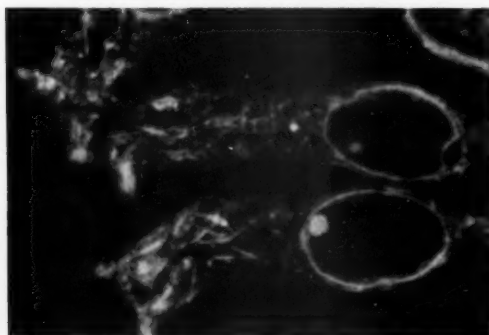


FIG. 1. Cylindrical cells from the endocervical mucosa, only slightly mucus producing. The nucleus is oval and basally located. In the cytoplasm there are filiform formations and only a few mucus clods on the apical end of the cell. Anoptal contrast, 3520 X.

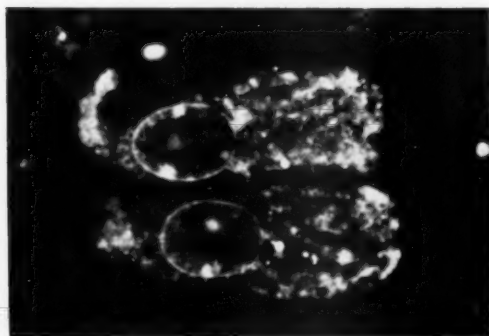


FIG. 2. Secreting cells in an advanced stage of mucus production. Nuclei are similar to those in Fig. 1. The cytoplasm is filled with coarse mucoid substances. The filiform formations have almost disappeared. The apical end of the cell is straight or slightly convex respectively. Anoptal contrast, 3080 X.

nuclei are oval-shaped and are basally located. One seldom sees in this stage of mucus production the round or oval nuclei slightly removed from the cellular base (Fig. 3). It would seem logical to assume that this picture corresponds to what is seen in endometrial cells in the early secretory phase. In the histological section one rather frequently finds this moving off the cellular base. I am not able, however, to put this feature into a significant correlation with the phase of the cycle, i.e., with the early secretory phase.

During the fully developed secretory activity the endocervical cells (Figs. 4, 5) take a more goblet shape. The secretory substances in the cytoplasm are more coarsely dispersed; the nucleus becomes more and more crescent-like and is pushed against the basal end of the cell. Between the nucleus and the upper membrane of the cell one finds a zone which is more or less free from mucus, similar to a vacuole. The apical end of the cell is not sharply defined due to mucus secretion which corresponds to the cytoplasm, stains in various red-violet shades and is cyanophilic only in the vicinity of the nucleus.

The average size of the mucus-producing cells is around 40μ and they are about 8μ thick. The fully developed goblet cells are somewhat shorter, but considerably wider.

As compared with the mucus producing cells the ciliated cells are definitely in the minority. In the fresh specimen one can easily find the ciliated cells even under low power, since the kinocilia show a lively movement even hours after extirpation of the uterus.

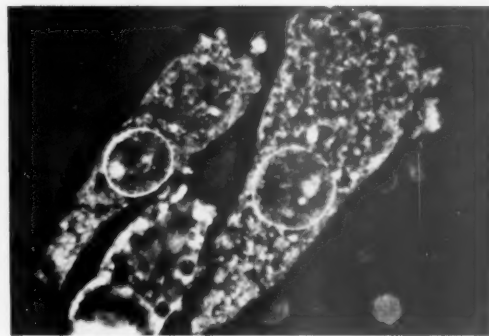


FIG. 3. Single mucus-producing cells show a roundish nucleus which is detached from the cellular base and moved towards the center of the cell. Anoptal contrast, 2100 X.

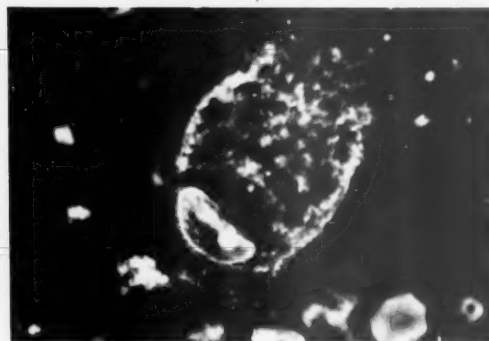


FIG. 4. Onset of the apocrine secretion; cell filled with cloddy mucus takes crescent shape and the nucleus is pushed towards the cell base. Anoptal contrast, 2700 X.

The ciliated epithelia (Figs. 6, 7) show a rather round nucleus which moved up from the cell base and is located almost in the center of the cytoplasm. Around the nucleus there



FIG. 5. Mucus-producing cell of goblet cell type. The shell-like nucleus lies on the basal end of the cell, the cytoplasm is filled with coarse mucoid substances. Apically from the nucleus there is a zone free from mucus. Anoptral contrast, 2330 \times .

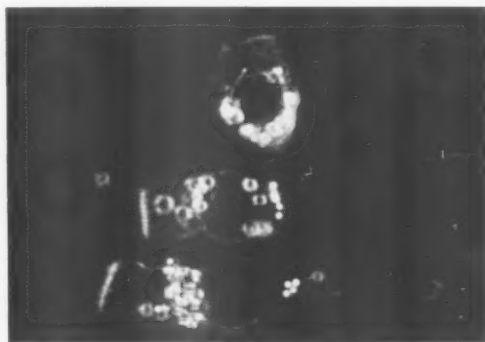


FIG. 6. Three ciliated epithelial cells from the endocervix. The round nucleus is found in approximately the center of the cell. The kinocilia originate from a basal knot line. Around the nucleus there are bright light reflecting droplets or secretions. Anoptral contrast, 2200 \times .

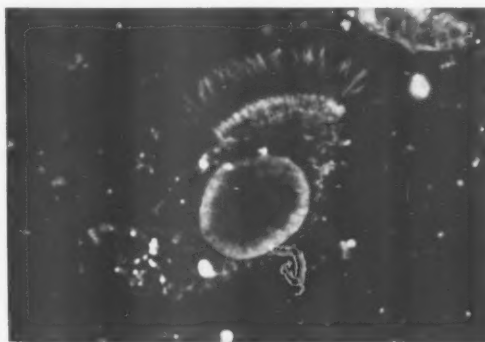


FIG. 7. In this slightly squeezed cell the ciliary lining is spread fan-shaped and the basal knot line is particularly well pronounced. Anoptral contrast, 2960 \times .

are almost always a number of markedly light-reflecting droplets of secretion which are, however, obviously not identical with the ones found in mucus-producing cells. The cilia arise from basal nodules. In properly stained smears (Papanicolaou) the row of basal knots and the cilia themselves stain brightly eosinophilic. The cytoplasm is cyanophilic except for a small area lateral and apical of the nucleus, which also stains eosinophilic (Fig. 8).

The size of the ciliated cells is essentially the same as the size of mucus producing cells. The average length of the cilia is 7 μ .

In order to obtain the correct impression of the mode of motion of the cilia it is necessary to observe many viable endocervical ciliated cells. The individual cilia move in one plane. In cells which are located on the glass slide in such a manner that their ciliary motion is parallel to the plane of the slide, their basal knots appear like a finely dotted line. The kinocilia strike in regular intervals towards the sides with a frequency of about 150 strokes per minute. The motions of the cilia seem quite forceful. The viscous mucus around the cell is vividly moved and often the entire cell moves around a central axis perpendicular to its longitudinal axis. The cilia appear like elastic rods, their ends oscillating after onset of the forceful strokes. In the cytoplasm of ciliated cells one may



FIG. 8. Some ciliated epithelia stained according to the Papanicolaou technique. In the upper right corner is a cyanophilic, superficial cell. Cilia and basal knots stain eosinophilic (darkly stained in the photograph). Between basal knots and the nucleus there is an eosinophilic zone. The remaining cytoplasm is cyanophilic. Oil immersion, 2300 \times .

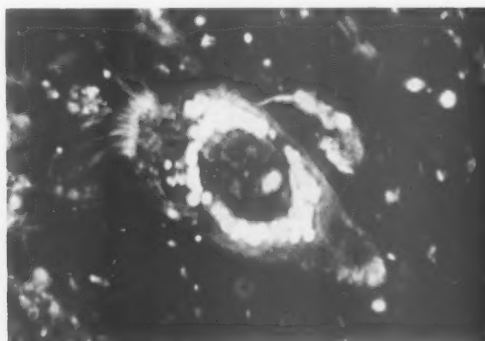


FIG. 9. In some ciliated cells one may faintly see the ciliary roots which extend from the basal knots toward a zone laterally located between the nucleus and the apical cell border. Anoptral contrast, 2900 \times .

see between the nucleus and the line of basal knots an area which is contracted and relaxed rhythmically, synchronizing with the ciliary stroke, similar to a systole and diastole. This area corresponds to the above mentioned site, which stains eosinophilic in the smear stained according to the Papanicolaou technique. Rarely can one faintly discern thread-like connections between this area and the basal knot line, corresponding to the so-called ciliary roots which transmit the motion of the contractile cytoplasmic substance to the cilia (Fig. 9).

During the systole of the described cytoplasmic area, one sees, under close observation, the stroke of the cilia to one side in addition to a minute lateral shortening of the distance between nucleus and basal knot line, in such a way as to result in a slight tilting of the apical end of the cell. I was unable to find ciliary motions which were more rapid to one side than to the other as described again and again in the textbooks. The back and forth strokes of the cilia take place at exactly the same speed and precisely the same rhythm. One may assume, however, that the motion caused by the cytoplasmic contraction may be more forceful than the one resulting from the cellular relaxation.

On ciliated cells which lie on the glass slides in such a way that the ciliary strokes are perpendicular to the plane of the slide, one can well recognize that the basal knot line embraces a mouth-like or tube-like cellular margin. One may clearly observe two moving lips during the ciliary motion, since one has only one half of the basal knot line in focus while the other half disappears either above or below the focal plane.

One also regularly finds individual typical mucus-producing cells with oval-shaped nuclei which are moved slightly off the cellular base with an intimated basal knot line and sometimes remnants of cilia. The significance of these cells (Fig. 10) will be discussed later.

In histological sections one regularly finds ciliated cells, whether we deal with a uterus of a woman in the reproductive age or that of a senile individual. Special staining procedures for demonstrating the cilia are not required. However, the histological specimen has to be fixed properly, to be handled carefully and thin sections have to be made from the material. The majority of the cells are mucus-producing. Single ciliated epithelial cells are intermingled between the mucus-producing cells of the surface and the glands as well (Fig. 11). Without that, any correlation to the phase of the menstrual cycle would be unrecognizable. Without following any apparent rule one may find on the superficial epithelium, as well as within the glands, small stretches covered entirely by ciliated epithelia (Fig. 12).

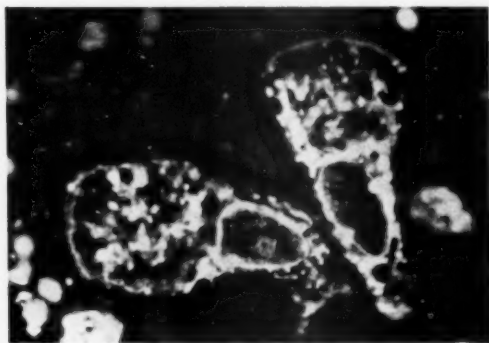


FIG. 10. Single, already mucus-producing, endocervical cells with remnants of basal knot lines and cilia. 2690 \times .

The skilled observer recognizes the ciliated cells in the histological section, even under low power, by means of nuclei which are moved off the cellular base and by the rather dark staining cytoplasm which is also evident with the regular hematoxylin-eosin stain.

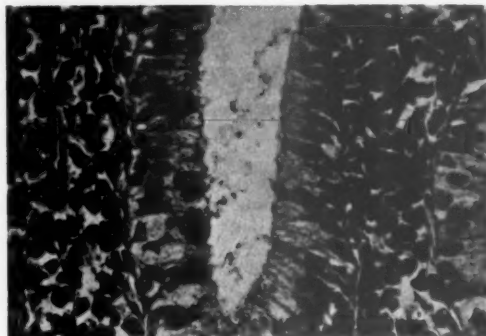


FIG. 11. A glandular tube lined by mucus-producing cells, with scantily intermingled ciliated cells. The cytoplasm is distinctly darker staining. Goldner stain, 880 \times .

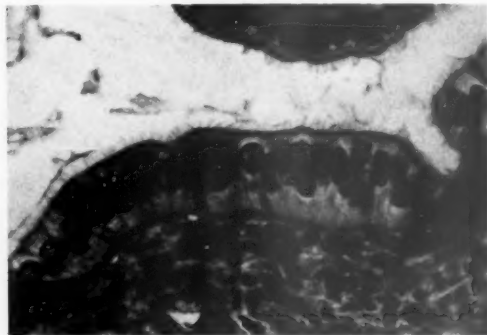


FIG. 12. Surface epithelium of the endocervix with glandular opening. All epithelia are ciliated, their nuclei are moved off the cellular base towards the interior of the cell. Hematoxylin-eosin, 960 \times .

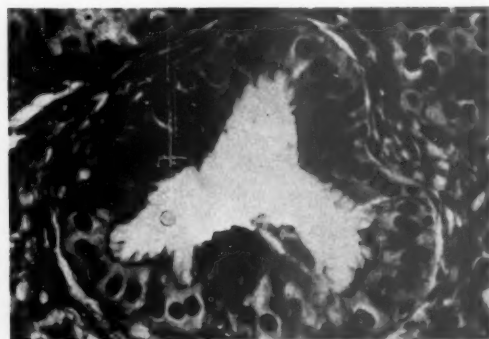


FIG. 13. A cervical gland from the portion of the endocervix close to the lower uterine segment. In this region there are frequently found single glands which are completely lined by cilia.

As a common change of the endocervical mucosa dependent upon the phase of the menstrual cycle, one finds, around the midcycle, nuclei which are moving away from the basal end of the cell and, during the early luteal phase, a slightly increased spiral formation of the glandular tubules. These changes, however, are so slightly pronounced that even the experienced observer is often unable to determine the phase of the menstrual cycle from observing the histological section. In senile women one finds, however inconstantly, a more or less pronounced atrophy of the endocervical mucosa.

DISCUSSION

As far as the cyclic changes in the endocervical mucosa are concerned, there are no secretory changes which could be comparable to the respective secretory changes in the endometrium, since the former is found in a constant stage of secretion. The secretory activity seems to reach a peak during the midcycle, and apparently decreases with increasing progesterone production. In every single slide throughout the menstrual cycle, however, one finds, in the endocervix, portions with relatively low epithelia which can easily be discerned as effete along the side of other sites where marked mucus production is present.

The hormonal stimuli causing the secretory activity seems to be estrogenic as was previously suggested in 1938 by Sjoevall (9). The progestational effect can be observed in the periglandular stroma of the endocervix, which may be increased to a well developed focal decidual reaction during pregnancy.

Of special cytological interest are the ciliated epithelia in the endocervix. One may well assume that here we deal with various functional stages of the very same type of epithelial cells. From the present observations it may be gathered that the ciliated epithelia are loosening their cilia and are being transformed into mucus-producing cells. The cellular features in Figure 10 can be interpreted in this way. The ciliated epithelia lie irregularly among the mucus-producing cells, and there seems to be no connection between their number and localization and the menstrual cycle, a fact which has been stressed by Stieve in 1927 (10) and also by Pananicolaou (6). The above observation seems interesting in that one almost constantly finds glandular tubules and also areas of surface epithelium in that portion of the endocervix adjacent to the lower uterine segment which display exclusively ciliated epithelium.

One may assume that the ciliated cells migrate from this position of the endocervix to the external os and that they loosen their cilia on the way more or less rapidly, being transformed into mucus-producing cells. The question, however, whether or not all mucus-producing cells derive from ciliated cells, remains open.

The function of the ciliated cells in the endocervix seems to be completely unknown. Frequently one can read about ciliary strokes that move faster in one direction than in the other. As already pointed out, such a phenomenon has never been observed in the present study. The cilia probably strike in one plane and in the direction from the fundus uteri to the external os. The stroke in one direction is likely to be more forceful than in the other; a difference in velocity of the two

movements, however, does not seem to exist. Stieve was opposed to the widespread belief that the undulation of the cilia is supposed to guide the spermatozoa which are compelled to swim "against the stream." If it were so, one should assume that the majority of them would lose their way hopelessly in the labyrinth of the tubes of the endocervical glands and disintegrate there. The opinion of Stieve (10) is also unlikely in that it is the function of the ciliated epithelia to avoid congestion of mucus in the endocervical canal and the glandular tubes. The ciliated epithelia are distributed too irregularly and are completely absent on large stretches so as to fulfill a task of that kind.

The only remaining satisfactory explanation would be that the function of the ciliated cells is to blend a specific secretory product into the mucus produced by the other cells. As already brought to evidence by the ordinary hematoxylin-eosin staining procedure, the ciliated epithelia do not contain the same product as the mucus-producing cells, since the cytoplasm of the ciliated cells stains clearly eosinophilic, whereas the cytoplasm of the mucus producing cells takes up very little of the eosin stain.

Using the staining method of Goldner, the difference in cytoplasmic staining reaction is even more pronounced, namely green with the ciliated cells and reddish-purple with the mucus-producing cells. Also with the Papanicolaou procedure there are remarkable differences in the staining reaction of the cytoplasm of the two types of cells; in other words, all important hints are that there are chemical differences in the respective secretions. As shown in the figures, these differences are also evident in the unstained cells of the fresh specimens in the form of coarsely dispersed mucus masses, in the mucus-producing cells as contrasted to the brightly gleaming droplets in the ciliated epithelia. As to the nature of this difference, histochemical studies alone could provide pertinent information.

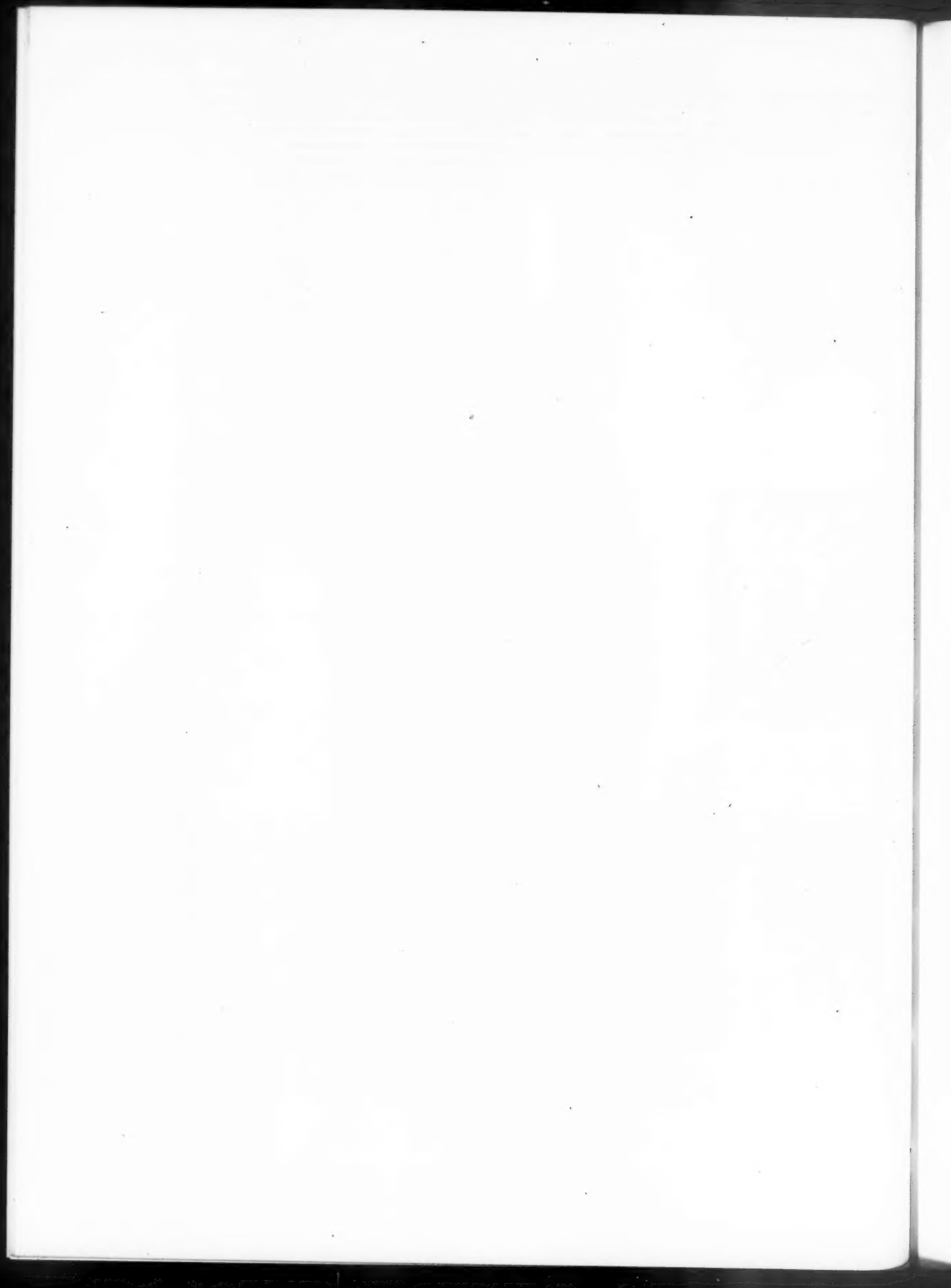
SUMMARY

In the uterine endocervix one finds ciliated cells in addition to a majority of cells consisting of highly cylindrical mucus-producing cells. Even hours after preparation of the smear and after immersion in the isotonic solution the viable ciliated cells show lively strokes of the cilia. The ciliary strokes take place uniformly back and forth in one plane with a frequency of about 150 strokes per minute. The motion of the cilia originates in a contractile cytoplasmic substance which is located eccentrically between the nucleus and the free end of the cell. As the sole phase of the movement, which has a determined direction, there is an inclination to one side of the ring-like free cellular end, which is covered by the basal knot line. This takes place during the contraction of the above cytoplasmic area. The function of the ciliated cells is probably to blend their secretory product into the mucus secreted by the mucus-producing cells. The secretion of the ciliated cells seems to be chemically different from that of the mucus-producing cells.

The distribution of the cells which seem to be merely different functional stages of the same cell type is completely irregular within the endocervix. The menstrual cycle is reflected only very faintly by cyclic changes of the endocervical epithelia.

BIBLIOGRAPHY

- BRABURN, G. B. and WEBB, C. F.: *Am. J. Obst. & Syn.* 62:997, 1951.
- GOLDNER, J.: *Am. J. Path.* 14:237, 1938.
- HOEHNE, O.: *Zbl. Gynakol.* 32:121, 1908.
- MANDL, L.: *Ztschr. Geburtsh. Gynakol.* 34:150, 1911.
- NOVAK, E.: *Gynecologic and Obstetric Pathology*. Philadelphia and London, 1952, Saunders.
- PAPANICOLAOU, G. N., TRAUT, H. F., and MARCHETTI, A. A.: *The Epithelia of Woman's Reproductive Organs*. Cambridge, Mass., 1948, Harvard University Press.
- PAPANICOLAOU, G. N.: *Atlas of Exfoliative Cytology*. Cambridge, Mass., 1954, Harvard University Press.
- SCHÜLLER, E.: *Mikroskopie* 10:335, 1955.
- SJOVALL, A.: *Acta obstet. et gynecol. Scandinav. (Suppl. 4)* 18:3, 1938.
- STIEVE, H.: *Ztschr. mikroskop.-anat. Forsch.* 11:291, 1927.
- TOPKINS, P.: *Am. J. Obst. & Gyn.* 58:645, 1949.
- WINKLER, H.: *Geburtshilflich-gynakologische Propädeutik*. Berlin, 1944, Springer.



THE SYMPOSIA BY CORRESPONDENCE OF ACTA CYTOLOGICA

INTRODUCTORY REMARKS

The Symposia of ACTA CYTOLOGICA are held entirely by correspondence and contain international discussions of scientific problems of interest to the exfoliative cytologist.

System for Selecting Subjects for Symposia: From recommendations received, the Editorial Office will draw up the list of subjects and will publish these subjects in ACTA CYTOLOGICA, under the heading FUTURE SYMPOSIA.

The final detailed program will be published in ACTA CYTOLOGICA immediately preceding the one where these topics are to be considered, under the heading, THE NEXT SYMPOSIUM.

Instructions for Authors: Each problem will be introduced by a *Main Speaker* or *Speakers*. These principal papers will then be considered by persons identified as *Discussants*. As a general rule, approximately 600 words each will be allocated for main papers and 200 words each will be allocated for the contributions of the Discussants. The Main Speakers will then be given the opportunity to make unlimited Closing Remarks.

Photomicrographs and tables may be reproduced: one full page for each principal paper and for the paper of the Discussant (maximum one-half page per contribution). The photomicrographs and tables should be submitted in glossy photographic prints, preferably in the size of 3 × 4 inches (i.e., 12 × 19 cm) and should show a proportional 10 μ scale on its reverse side. *Each figure should be accompanied by a comprehensive caption.*

The Discussants are requested to *strictly restrict their contributions to the discussion of the main papers*. Discussions which are not directly related to the main paper cannot be accepted. It is suggested that the Discussants prepare their contributions in such a manner that the reader may gain the impression of an actual round table conference.

The Closing Remarks of the Main Speakers should be limited to the answering of questions raised in the discussion and to other directly related information.

The Bibliography for the papers of both Main Speaker and Discussant should be organized in the same manner as in the American Journal of Obstetrics and Gynecology, at the end of the paper. *Every cited opinion or publication should have a reference in the bibliography.*

Deadline for Contributions: The Editorial Office will set deadlines for each written symposium. These will include:

1. deadline for agreements to contribute.
2. deadline for main papers.
3. deadline for discussions.
4. deadline for closing remarks.

LES SYMPOSIA PAR CORRESPONDANCE DES ACTA CYTOLOGICA

Les *Symposia par Correspondance* des ACTA CYTOLOGICA présentent des discussions internationales sur des problèmes scientifiques intéressant le cytologiste exfoliative.

Système du choix des sujets pour les symposia: En partant des propositions, et sous la rubrique: FUTURS SYMPOSIA, le bureau de rédaction dressera la liste des sujets principaux qui seront publiés dans les ACTA CYTOLOGICA.

Le bureau de rédaction établira le programme définitif et détaillé des discussions qui sera publié dans les ACTA CYTOLOGICA précédant immédiatement le symposium, sous la rubrique PROCHAIN SYMPOSIUM.

Recommandations pour les auteurs: Chaque sujet principal sera présenté par un Rapporteur Général ou des Rapporteurs. Ces mémoires principaux seront alors soumis aux Participants à la Discussion. En règle générale 600 mots seront accordés aux Rapporteurs des sujets principaux, et, 200 mots aux Participants à la Discussion. Les Rapporteurs Généraux pourront clôturer les discussions par un nombre illimité de remarques.

Des microphotos et graphiques pourront être reproduits à raison d'une page entière pour chaque sujet principal et une demi page au maximum pour les discussions. Les microphotos et les graphiques doivent être présentés sur du papier brillant, de préférence dans le format 12 X 19 cm. *Chaque figure devra être accompagnée d'une légende explicative précise.*

Les membres et invités prenant part aux discussions sont invités à *limiter strictement leurs interventions aux discussions des sujets principaux*. Des discussions qui n'ont pas de rapport direct avec le sujet principal *ne pourront être acceptées*. Il est recommandé que les discussions soient rédigées d'une manière telle que le lecteur ait l'impression d'assister à une discussion réelle de table ronde.

Les Remarques de Clôture du Rapporteur Général devront se limiter à la réponse aux questions soulevées dans les discussions et aux autres informations éventuelles ayant un rapport direct avec le sujet.

La bibliographie des rapports et discussions devra être rédigée de la même manière que celle de l'American Journal of Obstetrics & Gynecology et figurer à la fin du texte. *Chaque opinion ou publication citée dans le texte doit avoir sa référence dans la bibliographie.*

Dates limite pour les collaborations: Le bureau de rédaction, fixera des dates limites comprenant:

1. un délai pour l'acceptation des collaborations,
2. un délai pour les sujets principaux,
3. un délai pour les discussions,
4. un délai pour les remarques de clôture.

DIE SCHRIFTLICHEN SYMPOSIEN DER ACTA CYTOLOGICA

Die schriftlichen Symposien der ACTA CYTOLOGICA befassen sich auf internationaler Basis mit wissenschaftlichen Problemen, die für den Exfoliativ-Zytologen von Interesse sind.

System der Thema-Auswahl für die Symposien: Die Schriftleitung stellt auf Grund von Thema-Vorschlägen eine Liste von Haupt-Themen zusammen, und gibt diese Liste unter dem Titel ZUKÜNFTIGE SYMPOSIEN bekannt.

Die Schriftleitung bereitet das Programm mit allen Einzelpunkten vor, und veröffentlicht dieses Programm in dem Heft, das dem betreffenden Symposium vorausgeht, unter dem Titel DAS NÄCHSTE SYMPOSIUM.

Instruktionen für Autoren: Jedes Thema wird von einem oder mehreren Referenten behandelt. Diese Referate werden dann von Diskussions-Vortragenden besprochen. Im allgemeinen werden Referate auf etwa 600 Worte beschränkt, und Diskussions-Vorträge auf 200 Worte. Die Referenten erhalten dann die Gelegenheit, Schlussbemerkungen ohne Wortzahlbeschränkung zu machen.

Mikrophotographien und Tabellen können abgedruckt werden: eine Ganzseite kann Referenten und eine halbe Seite Diskussionsvortragenden für Abbildungen zur Verfügung gestellt werden. Die Photographien sind auf Hochglanzpapier, und möglichst in der Grösse 12×19 cm erbeten und soll ein proportionales 10μ Zeichen auf der Rückseite haben. *Jede Abbildung muss von einem erklärenden Untertitel begleitet sein.*

Die Diskussionsvortragenden sind gebeten, sich in ihren Beiträgen *streng an das Hauptreferat zu halten*. Diskussionsbeiträge, die sich nicht an das Hauptthema halten, *können nicht berücksichtigt werden*. Es wird vorgeschlagen, dass die Diskussionsvorträge in einem Stil abgefasst sind, dass der Leser den Eindruck gewinnt, als ob es sich um eine Diskussion am runden Tisch gehandelt hätte.

Die Schlussbemerkungen der Referenten sollen sich nach Möglichkeit auf die Beantwortung von Diskussionsfragen beschränken.

Die Bibliographie der Referate und der Diskussions-Vorträge sollen *am Schluss* der Beiträge nach dem Muster der Bibliographien im American Journal of Obstetrics and Gynecology aufgeführt werden. *Jede zitierte Ansicht oder Publikation muss eine Referenz in der Bibliographie haben.*

Termine für Beiträge: Die Schriftleitung setzt Termine für die Schriftlichen Symposien fest. Die folgenden Termine werden bekanntgegeben:

1. Termin für Erhalt der Beitrags-Zusagen,
2. Termin für Erhalt der Hauptreferate,
3. Termin für Erhalt der Diskussions-beiträge.
4. Termin für Erhalt der Schlussbemerkungen.

SIMPOSIUM ESCRITO DE ACTA CYTOLOGICA

El simposium escrito de ACTA CYTOLOGICA contiene discusiones internacionales sobre problemas científicos que son de interés para el citólogo exfoliativo.

Sistema de selección de materias para el simposium: Con sugerencias recibidas, la oficina editorial confeccionará una lista de los temas más interesantes, lista que será publicada en ACTA CYTOLOGICA con dos números de anticipación a la fecha de su posible publicación, bajo el epígrafe de "SIMPOSIUM FUTUROS."

La Oficina Editorial confeccionará y publicará una lista detallada del programa de la discusión en el número de ACTA CYTOLOGICA inmediatamente anterior a aquel en que han de ser incluidos los temas, bajo el epígrafe de: EL PROXIMO SIMPOSIUM.

Participación en el Simposium Escrito: No habrá restricción alguna sobre el número de puntos de discusión en los que cualquier autor desee participar.

Instrucciones a los Autores: Cada problema deberá ser presentado por un ponente o ponentes. Estos trabajos principales serán entonces discutidos por los comunicantes. Como regla general, se permite un máximo de 600 palabras para los trabajos principales y 200 palabras para las contribuciones de los comunicantes. Al ponente principal se le da la oportunidad de hacer rectificaciones finales ilimitadas.

Pueden reproducirse microfotografías y tablas: una página por cada trabajo principal y un máximo de media página por discusión. Las microfotografías y tablas deberán enviarse en forma de copias fotográficas amplias. A ser posible de 3 x 4 pulgadas (12 x 19 cms). *Cada figura deberá acompañarse de su correspondiente leyenda.*

Se suplica a los comunicantes *ajustar estrictamente sus comunicaciones a la discusión de los trabajos principales*. Las discusiones que no estén directamente relacionadas con el trabajo principal *no podrán ser aceptadas*. Se sugiere que los comunicantes realicen sus contribuciones de manera tal que el lector tenga la impresión de estar ante una verdadera mesa redonda.

Las rectificaciones finales de los ponentes deberán limitarse a contestar las preguntas aparecidas a lo largo de la discusión así como a otras directamente relacionadas con el tema.

La bibliografía, tanto de las ponencias como de las comunicaciones deberá redactarse de la misma forma que figura en el American Journal of Obstetrics and Gynecology, *al final del trabajo. Toda opinión o publicación citada deberá tener su correspondiente referencia en la bibliografía.*

Fechas para las contribuciones: La Oficina Editorial, fijará fechas límite absolutas para cada simposium escrito. Estas incluirán:

- 1°. Fecha límite para acuerdo de contribución,
- 2°. Fecha límite para las ponencias,
- 3°. Fecha límite para las discusiones,
- 4°. Fecha límite para las anotaciones finales.

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Symposium

RADIATION CELL CHANGES

DEFINITION OF RADIATION RESPONSE ON NORMAL SQUAMOUS CELLS (RR CELLS)

RUTH M. GRAHAM

Buffalo, New York, U.S.A.

Cellular Radiation Response may be defined as definite morphological changes occurring in the benign squamous cells under the influence of ionizing radiation. These morphological alterations occur in the basal, intermediate and superficial cells. The changes are vacuolization of the cytoplasm, increase in size of both nucleus and cell, definite nuclear changes, and the occurrence of multiple nuclei.

Fig. 1 illustrates four vacuolated basal cells. It is apparent from the photomicrograph that the cytoplasmic vacuoles vary considerably in size. In the cell on the left the vacuoles are extremely fine, while the adjacent cell has two large vacuoles, one overlying the nucleus. The larger basal cell in the center has many vacuoles varying from extremely small ones to the large vacuole above the nucleus. In contrast, the cell on the right has only one vacuole but it occupies more than half the cell. The common type of vacuolization encountered in the intermediate cell is illustrated in Fig. 2. The vacuoles are small and are distributed throughout the cytoplasm. Vacuolization occurs occasionally in the superficial cells but not often enough to be a predominant feature of radiation response.

The increase in cell size is perhaps the most striking of the morphological alterations. In Fig. 3 size increase in a basal cell is illustrated. Notice that this one cell occupies almost the entire field. It should be compared to Fig. 1 where four basal cells occupy the same area. The nucleus has increased in size as well as the entire cell, but it is still finely granular. This cell is identified as a radiated basal cell because of its oval shape and cytoplasmic nuclear ratio. The increase in cell size may be extraordinary as exemplified in Fig. 4, illustrating increase in size in an intermediate cell. This cell was so large it was impossible to photograph it in its entirety. The shadow of a superimposed normal size intermediate cell may be seen just below the large nuclei, an indication of the usual size of intermediate cells. This cell is recognized as an intermediate one by the large amount of cytoplasm present, the vesicular nuclei and the squared off cytoplasmic borders. Fig. 5 illustrates the phenomenon of size increase in a superficial cell. It is similar to that in the intermediate cell except for the presence of a small pyknotic nucleus, identifying it as a superficial cell.

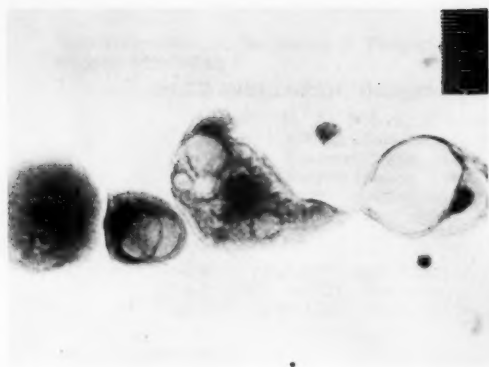


Fig. 1

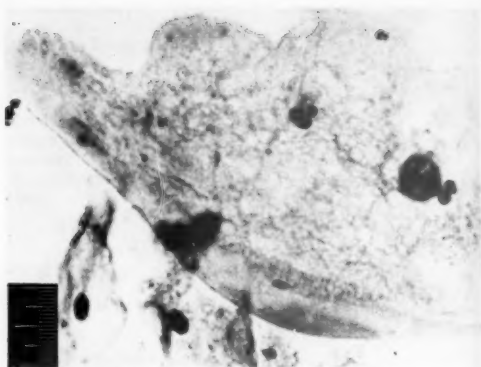


Fig. 2

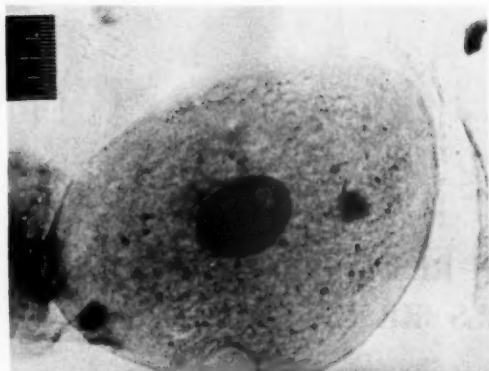


Fig. 3

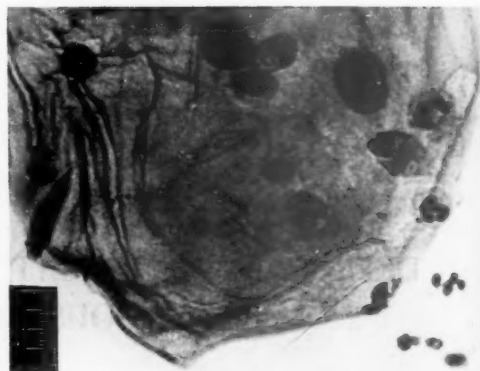


Fig. 4

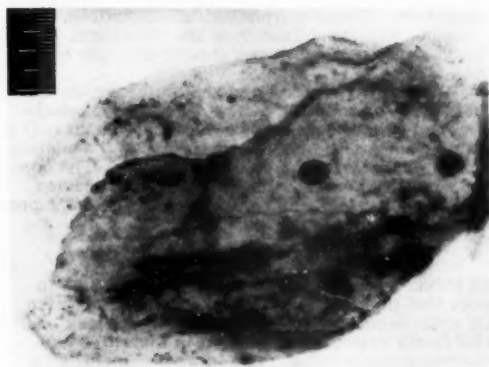


Fig. 5



Fig. 6

The most prominent nuclear change is seen in Fig. 6, illustrating the wrinkling of the nuclear surface. Large wrinkles cover the surface of the nucleus. This change is only in cells whose nuclei are already enlarged. It appears that the nucleus has more surface area than can be smoothly expanded in

the space it occupies, so that extensive wrinkling of the surface takes place. This change is identical in intermediate cells, as may be seen in Fig. 7. Since by definition the superficial cell has a completely pyknotic, structureless nucleus, no such change is seen in the superficial cells.

The presence of multiple nuclei needs little comment, Figs. 8, 9 and 10 illustrate this phenomenon in a basal, an intermediate, and a superficial cell. These cells exhibit none of the other changes due to radiation.

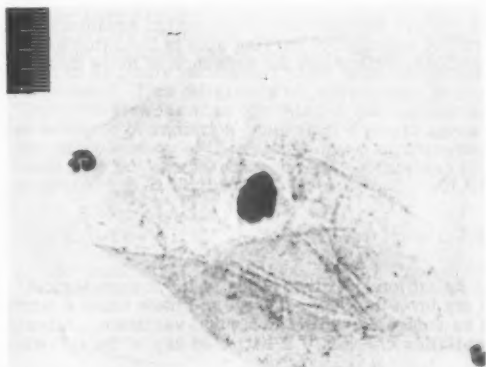


Fig. 7

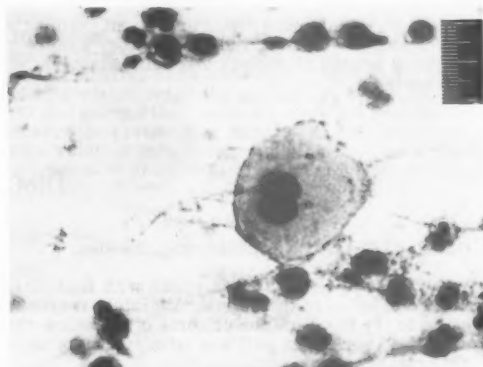


Fig. 8

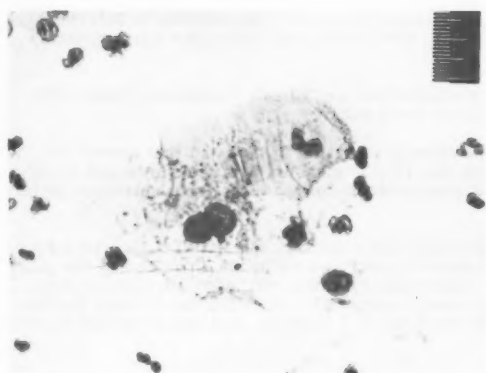


Fig. 9

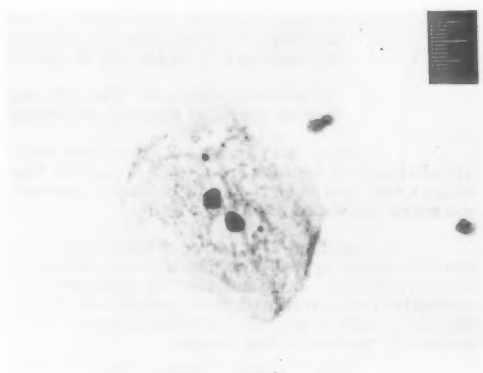


Fig. 10



Fig. 11

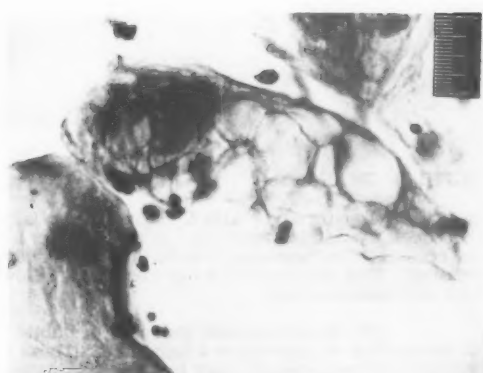


Fig. 12

Occasionally a cell will show two, three or even all four changes. Fig. 11 illustrates an enlarged basal cell with prominent vacuolization, beginning wrinkling of the nucleus plus another phenomenon frequently encountered, phagocytosis. Contained in one of the large vacuoles is some unidentified phagocytosed debris.

The basal cell illustrated in Fig. 12 has all four of the characteristic radiation changes. The vacuolization is extensive and resembles bubbles. Both the nuclei and the cell are increased in size. There is wrinkling of the nuclear surface. Two nuclei are present.

In summary, four prominent cellular alterations are encountered in the benign squamous cell in response to ionizing radiation. They are the presence of vacuoles of varying size in the cytoplasm, increase in size of nucleus and cell, wrinkling of the nuclear surface and the presence of more than one nucleus.

DISCUSSION

OLLE KJELLGREN, Gothenburg, Sweden:

I agree in all essentials with Ruth Graham's definition and description of the morphological characteristics of the cellular radiation response. In my investigations, however, I have found it worthwhile to try to define the criteria of radiation changes as contrasted with the normal variation. A benign exfoliated squamous cell was interpreted as showing radiation changes if it satisfied any of the following criteria:

- a. Size increase: A cell is enlarged if its major axis exceeds 35μ in basal cells or 68μ in intermediate or superficial cells.
- b. Nuclear changes: Two or more nuclei are present in the cell; the nucleus is deformed and has acquired a wrinkled or folded appearance; the nucleus has undergone karyorrhexis; the nucleus of a basal cell is pyknotic.
- c. Cytoplasmic changes: The cell exhibits vacuolization or "fibril" formation; basal cells display modified staining properties; the cell has a bizarre shape.

Comment to a: The dividing-line between the normal variation in size and that caused by irradiation was decided from 5000 measured basal cells and 10,000 measured intermediate and superficial cells, which showed that about 2-3 percent of the size variation of the cells from untreated patients exceeded the above mentioned limits.

Comment to b and c: Even before irradiation there are a certain number of cells with such a morphological appearance that it would have been registered as showing radiation changes had the patient been irradiated. In calculating the frequency of such "unspecific radiation changes" 10,000 squamous epithelial cells from untreated patients at different ages were examined. In individual smears the frequency of such unspecific radiation changes varied between 0 and 8.5 percent, and was less than 4 percent in 80 percent of the smears.

Bibliography

1. Kjellgren, O.: Acta Radiol. Suppl. 168, 1958.

CLOSING REMARKS

RUTH M. GRAHAM:

I would agree with Kjellgren that it is possible to determine by cell measurements whether or not a cell shows size increase. A study in our laboratory (1) on the increase in cellular size showed that intermediate and superficial cells larger than 66μ in diameter could be considered as showing an increase in size. This is close to the 68μ mentioned. However, in doing our routine radiation counts we seldom use cell measurements.

We do not consider that a cell exhibits radiation response if the nucleus shows karyorrhexis or if the nucleus is pyknotic in a basal cell. These changes seem to me to be such non-specific degenerative changes and are seen so often in cases with no radiation that to interpret them as evidence of radiation would be misleading.

In the great majority of cells the "fibril" formation is associated with vacuolization so that we have not classified this as a separate cell change. I am not quite certain what is meant by a "modified" staining property. We do not consider any change in staining quality as an effect of radiation.

Though presumably this symposium was to be limited to the effects of radiation, I think that Kjellgren's comment that "unspecific radiation changes" occur in from 0 to 8.5 percent of the cells deserves comment. I would disagree completely with this statement. When a series of smears taken before and during treatment were read blindly - that is, the cytologist had no knowledge of whether there was one control smear, six control smears or none - it was found that cells interpreted as showing radiation response were present in about 40 percent of the smears from patients with invasive carcinoma of the cervix before treatment, and in numbers greater than 10 percent. In reviewing the slides in which radiation response was interpreted as having been present before any radiation was given, the cells were found to be finely vacuolated basal cells which have since been called cells showing the "Sensitization Response." This difference of opinion may be explained by a statement in the monograph by Kjellgren(2), "Vacuoles smaller than one third of the nuclear diameter and perinuclear vacuoles were not counted as evidence of radiation changes." I would agree concerning the perinuclear vacuole, but we consider fine vacuolization of the cytoplasm as an important change which occurs during radiotherapy and a change which may be seen fairly frequently before any treatment and is of prognostic significance when it occurs in numbers greater than 10 percent.

Bibliography

1. Graham, R. M. and Goldie, K. R.: Cancer 8:71, 1955.
2. Kjellgren, O.: Acta Radiol. Suppl. 168:49, 1958.

MORPHOLOGY OF THE IRRADIATED SQUAMOUS EPITHELIAL CELLS (RR CELLS)

ARTURO ANGEL ARRIGHI

Buenos Aires, Argentina

In normal exfoliated cells of the vaginal epithelium the ionizing radiation induces two types of morphological changes: progressive and regressive.

The former, the first to be present after the radium is removed, is characterized by enlargement of the cells to two to three times (or even more) the size of the normal cells. This alteration occurs in the parabasal, intermediate and superficial cells. The nuclei of the first two are also larger than normal, so that the normal nuclear-cytoplasmic ratio is preserved.

This change is, in our opinion, the most peculiar of the morphological alterations encountered in benign squamous cells as a result of radiation. Sometimes one observes bizarre-shaped cells (tadpole cells, butterfly-like cells, etc.).

The regressive type of cellular changes consists of degeneration or necrobiosis of the irradiated cells. It occurs after the increase in size has taken place, but because of the persistence of the latter, both changes are practically superimposed.

As a result of the alteration of its colloidal structure, the cytoplasm presents varying amounts and sizes of vacuoles. The fact that the cytoplasmic vacuoles are observed nearly exclusively in the parabasal and intermediate cells may be due to the fact that these elements disintegrate and disappear before they become superficial cells.

The nuclei also suffer the effect of radiation, and we can observe karyorrhexis, wrinkling of the nuclear membrane, irregular lobulation, abnormal mitosis, etc.

RUTH M. GRAHAM

Buffalo, New York, U.S.A.

There are two morphological alterations of squamous epithelial cells in addition to those described in the definition. First, an occasional cell may be extremely bizarre in shape. There is a multiplicity of forms such as tadpole, ameboid, spindle, etc. Originally we considered bizarre forms as a separate change. However, on differential counts such cells only account for 1 to 2% of the radiation change. Furthermore, such cells almost without exception have one of the more common changes such as vacuolization or size increase. Therefore, though these cells are perhaps the most striking of any encountered, we do not consider them as a separate entity.

Occasionally the basal cells will show a difference in staining reaction. They will have a thick, dark, cyanophilic border and around the nucleus a heavy yellow deposit. This yellow deposit often appears brittle since it may have definite cracks running across the surface. This alteration in staining may be encountered in atrophic smears from patients who have received no radiation, so that this change is not a specific characteristic of a cellular reaction to radiation.

In patients whose benign cells are reacting to radiation, small histiocytes appear as a prominent feature of the picture. In some instances they may be so numerous that they outnumber the leukocytes. In the latter part of radiation therapy and post-radiation, giant histiocytes are common.

Though our particular interest has been in the benign cells reacting to radiation, it should be pointed out that the malignant cells undergo identical changes under the influence of ionizing radiation. They may become extremely vacuolated, increase in size and present wrinkling of their nuclei. Multiple nuclei are a common finding.

HORST SMOLKA

Kiel, Germany

Cellular alterations in cytologic smears caused by ionizing radiation attract our attention: particularly a considerable vacuolization of the parabasal cells and an enormous increase in the size of the superficial cells. These two characteristics - if they are marked - may be considered as specific for a radiation response.

The cells of the deeper layers of the epithelium may contain vacuoles of very different sizes. Sometimes the cell has a vesicular, ballooned appearance. The cell border may yield to the pressure of the large vacuoles, which in this case determine the shape of the entire cell. The nucleus is often pressed toward the cell border. The origin of the cell from the parabasal layer can often be recognized by its characteristic vacuolization alone. In case of a high radiation effect the cell may become six to eight times its original size. Cell deformities are frequent, and the cells sometimes exhibit protrusions resembling pseudopodia. The cellular borders often become indistinct, and the cells may fuse together. The cytoplasm, which normally is cyanophilic, frequently stains brown, reddish, or orange, generally becomes darker and shows a turbid homogenization. The nuclear alterations are, in the main, a decrease of the structure, shrinking and deformation of the nucleus, or, frequently, karyorrhexis. An increase in the size of the nucleus is encountered occasionally. Radiation treatment often leads to multinucleation.

The intermediate cells also exhibit an increase in size following radiation. Deformities are not so striking since the cell shapes vary greatly by nature. However, the surface of the cell often appears to be transversely by ridges and wrinkles. The cytoplasm frequently stains brown and orange. Vacuolization is often present; however, it is not as marked as in the parabasal cells. The predominant alterations of the nucleus are a decrease of the structure, nuclear deformities, and shrinking or pyknosis.

As for the superficial cells, the most striking point is the enlargement of the cell, which may increase to such a degree that it is three to six times (in extreme instances even eight times) its original size. The cell shape generally remains relatively constant. However, there is an increase in foldings on the flat cell body. Vacuoles are only rarely encountered. If they are present, they are very small. The staining of the superficial cells in particular tends to be eosinophilic after radiation. Multinucleation is often observed also as in the intermediate cells. Occasionally one comes across pictures of nuclear divisions showing knot-like figures of secondary nuclei, which are connected with each other due to pseudoamitoses. The nuclear alterations also consist of enlargement, decreases of structure, folding, shrinking and pyknosis. Karyorrhexis, which is observed frequently in the cells of the deeper layers, is rather rare in the superficial cells. In cases of an enlargement of the nucleus or the cell the nuclear-cytoplasmic ratio generally remains constant.

All these alterations which have been mentioned with a view to benign cells may, in principle, also occur in tumor cells. As the shapes and structures of these, by nature, are not typical, but very considerably, it is often very difficult to judge the alterations following radiation. There is hardly any guiding principle which might support the comparison with any reliability. Nevertheless, an increase in the size of the cell and the nucleus, and a considerable vacuolization as well as deformation of the cell are frequently striking. The cells show an increased tendency toward plasmolysis and fusing together.

The alterations of the benign and the tumor cells are accompanied by a considerable augmentation of leukocytes and in most cases also of histiocytes. The epithelial cells or their fragments are often closely covered by them, or the cytoplasm of the cell has been invaded by leukocytes, singly or in groups. This appearance of phagocytosis, as well as the above mentioned alterations of the cell shapes and structures, are denoted "Intermediärreaktion," while the term "Finalreaktion" has been chosen to characterize the processes of the entire cell destruction such as plasmolysis, karyolysis, karyorrhexis, etc., in which the whole picture eventually becomes clear (Mohr, Angel).

Bibliography

1. Angel, H.-W.: *Mikroskopie* (Wien) 10:221, 1955.
Zschr. Geburtsh. (Stuttgart) 146:11, 1956.
2. Besserer, G. and Smolka, H.: *Strahlentherapie*, 89:442, 1953.
3. Graham, R. M.: *Surg. Gyn. Obstet.* 84:153, 1947.
4. Graham, R. M. and Graham, J. B.: *Cancer* 8:59, 1955.
5. Mohr, H. J.: *Gynäkologische Zytologie* (H. Runge) Dresden, 1954, Steinkopff.
6. Smolka, H. and Soost, H.-J.: *Grundriss und Atlas der gynäkologischen Cytophysiologie*.
Stuttgart, 1956, Georg Thieme.

ERICA WACHTEL
London, England, U.K.

Cells exposed to radiation show damage manifested by nuclear and cytoplasmic changes. These alterations in cellular appearance are, in my opinion, not specific changes occurring only after exposure to radioactive rays, but may also be present, though admittedly to a lesser degree, in patients who have not received radiotherapy. In other words, the observed morphological alterations are probably due to unspecific degenerative processes following the use of a powerful destructive source. The interpretation of these changes is complicated by two factors:

1. The material obtained by vaginal aspiration contains benign irradiated cells, possibly abnormal cells derived from areas adjacent to frankly malignant tissues and irradiated tumor cells. It is questionable whether or not a clear distinction of these cells as to their origin can be made.
2. Not all the observed cellular changes present are necessarily a direct effect of irradiation, e.g., it is very probable that the presence of multiple nuclei in a cell is the result of reactive inflammatory processes coexisting with the tumor or following excessive tissue destruction by radiation.

The cytological changes in the nuclei of benign irradiated cells are identical with those described in histological sections of irradiated tissue and comprise shrinkage of nuclei (best observed in basal cells), karyorrhexis and karyolysis. In contrast to histology, abnormal mitoses are rarely seen in smears. Multiple nucleation is a frequent finding, but, as stated above, it is doubtful if this feature should be regarded as a direct radiation effect.

Changes in the cytoplasm of benign irradiated cells include:

1. excessive vacuolization (taking the form either of one large vacuole filling the cell body and displacing and compressing the nucleus, or of a fine honeycomb-like vacuolization.
2. swelling of the cell body and nuclear substance, greatly enlarging and distorting the cellular outlines.

Malignant cells show similar changes after irradiation. The nuclei may enlarge and become increasingly hyperchromatic; vacuoles may appear within nuclear substance, or occasionally karyorrhexis may be demonstrated. Cytoplasmic vacuolization and enlargement of the cell itself is often present.

In addition to the changes occurring in individual cells, there are also changes concerning the total smear appearance. The smear patterns have been described as having "the radiated look" (Hannah Peters), i.e., they are "dirty" smears showing a heavy leukocytosis, many histiocytes and a "state of unrest" in the epithelial cells; there is marked anisocytosis, anisonucleosis and a great variation in staining properties of cytoplasm and nuclei.

DISCUSSION

LUDWIG von BERTALANFFY, Topeka, Kansas, U.S.A. and FELIX D. BERTALANFFY, Winnipeg, Manitoba, Canada:

The acridine orange (AO) fluorescence method (1) allows an easy (application of only one dye) and rapid (time for processing: 6 minutes; average time for screening: 3 minutes) recognition of morphologic and cytochemical criteria. The morphology of irradiated cells described in other sections of this Symposium can easily be read in the AO pictures: increase in size and pleomorphism of cells and nuclei; bizarre shapes and "ballooning"; vacuolation of the cytoplasm (from mesh-like or honeycomb appearance to single large vacuoles occupying most of the cytoplasm); multinucleation; multiple nucleoli; karyorrhexis and karyolysis; heavy infiltration with leukocytes resulting in "dirty" smears; etc.

The opinion that the changes observed in irradiated cells are due rather to unspecific degenerative processes than to a specific radiation response appears to be plausible. However, as one would expect, the number of cells showing such degenerative changes induced by irradiation seems to be increased.

The AO method recommends itself for its rapidity and simplicity, making possible immediate diagnosis with a reliability equal to that of conventional methods. It further provides cytochemical characteristics (see this Symposium) in addition to morphologic criteria. Follow-up studies of radiation cases with the AO method are presently carried through and will be presented elsewhere.

EMMERICH von HAAM, Columbus, Ohio, U.S.A.:

I agree with Ruth Graham's observations concerning the percentage distribution of the bizarre forms of radiation cells. To us the giant histiocytes and giant fibroblasts are typical cells showing good

mesenchymal radiation reaction. I cannot quite agree with Arrighi's contention that an enlargement of the cellular elements represents a progressive change. This must be proven by cytochemical rather than morphological methods. Erica Wachtel stresses the difficulty in differentiating between radiation cells and the dyskaryotic cells found in areas adjacent to the carcinoma. It is my opinion that of all the changes quoted, vacuolation of the cytoplasm is perhaps more characteristic for an acute radiation effect than any nuclear changes described. This observation also holds true for the study of radiation effects in malignant cells in which vacuolation of the cytoplasm plays an important role. Smolka's multinucleation is only encountered occasionally in our material.

ANDRÉ PAGÈS and J. MONTEIL-SEURIN, Montpellier, France:

An important point which has not been outlined in the main papers is the period of time elapsing before the first radiation changes appear.

Even if the morphology of irradiated cells changes little, according to the therapeutic method employed, the type of therapy does influence the moment when these radiation changes occur. Let us begin with the description given by Ruth Graham and compare them, as we did, with smears from irradiation therapy with cobalt 60. It becomes evident that there are differences as to when cell changes begin. Thus, for patients treated only with cobalt 60, staining properties begin to intensify at the 20th day of therapy, but is not a constant finding before the 20th day. The enlarged cells appear on the 16th day, cytoplasmic vacuolization is constant after the 20th day and the presence of intracytoplasmic polynucleation is also a constant finding after the 22nd day of therapy.

In patients undergoing combined radium-cobalt therapy, two cases may be encountered.

1. Initial treatment with cobalt with four to five successive repeats of radium-cobalt combined:

The increased staining intensity appears much earlier (around the 12th day). The enlarged cells are a constant finding only much later (22nd to 36th day), and they show a second peak between the 48th and the 60th day. Vacuoles are visible after the 13th day. The intracellular polynucleation appears with the same delay as the above. On the contrary, when the treatment does not consist of anything but a single cobalt application, followed by only a single radium application, the changes in the staining affinity are very retarded and occur around the 30th day; the time of appearance of the other signs, however, vary slightly.

2. Initial treatment with radium followed by series of four, three or two repeats of combined radium-cobalt application:

Under these conditions the cellular changes appear in the following averages: change in staining intensity appeared after the 6th day and was constant after the 12th day, enlarged cells appeared between the 9th and 50th day, cytoplasmic vacuolization from the 13th day on and intraplasmic polynucleation after the 25th day.

Besides these considerations of the time factor, three morphological details seem important to us:

With cobalt 60, the vacuolization, which seemingly occupies the entire cell, appears as an extensive loss of substance extended almost over the entire cell. The enlarged cell seemed to us more frequent and, above all, more hypertrophic than with the conventional radiotherapy or radium therapy. On the other hand, the bizarre forms seemed to us rare as retarded with this therapy.

Hence, we think it useful to take into consideration the kind of irradiation therapy before establishing a prognosis by means of exfoliative cytology.

CLOSING REMARKS

ARTURO ANGEL ARRIGHI:

We don't agree with the statement of von Bertalanffy and Bertalanffy about the interpretation of RR changes as unspecific degenerative processes. We think that they are specific, although not constant, modifications produced by the actinic therapeutic agent.

We cannot answer Pagès and Monteil-Seurin because all our patients have been treated with the same technique (Paris, Curie Institute).

Dr. von Haam will have to excuse our use of the term "progressive"; we gave only a descriptive, very limited meaning.

RUTH M. GRAHAM:

To Dr. von Haam: I would agree that vacuolization of the cytoplasm is the most characteristic effect of acute radiation response. We have some preliminary evidence to suggest that it is the most critical cellular change. We have found that multinucleation without any other change is encountered infrequently and is found just as often in the good response to radiation as in the poor response.

To Drs. von Bertalanffy and Bertalanffy: I have had no experience with the acridine orange fluorescence method, so I am unable to comment on its merits or disadvantages in studying radiation smears.

To Drs. Pagès and Monteil-Seurin: I cannot tell from this discussion whether the authors are using telecobalt or brachycobalt; since the dosage employed is not mentioned, it is impossible to comment on their observations. I have not seen any differences in the cytologic responses between intracavitary radium or cobalt with the Stockholm technique. There is no difference in response at various energy levels of x-radiation. I have studied patients who have received x-radiation delivered at 200kv, 400kv, 1000kv, 2000kv and 3000kv and all showed similar cytologic findings falling into poor and good response groups.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

CYTOCHEMISTRY OF IRRADIATED CELLS

PIERRE HAOUR AND CLAUDE CONTI

Lyon, France

Qualitative and quantitative cytochemical changes in irradiated cells can be studied by the Feulgen reaction with the histophotometric technique which has been proposed for detecting cancer cells (1, 2, 3).

Our study concerned cervical cells and especially transitional cells, which are usually found in numerous clusters in irradiated smears. They appear as triangular or star-like small cells sometimes with elongated cytoplasm. With the Papanicolaou stain the cytoplasm often shows a blood-red color. In the first month after irradiation the vesicular pattern of the nucleus is noticed, with the chromatin appearing both granular on a clear background and condensed in a dark rim along the nuclear membrane. One or several small hyperchromatic nucleolar dots are seen.

This feature is, however, not specific for irradiated cervical smears and may be encountered in vascular troubles of the cervical mucosa.

Nuclear patterns when stained with Feulgen reaction are more characteristic. Only DNA is stained in the nucleus, the intranuclear appearance being more distinct than with the Papanicolaou stain.

We have been studying 14 patients treated by radium therapy who were followed during periods of time ranging from 4 to 64 months.

The following changes were noticed after irradiation:

1. Nuclear chromatin of benign irradiated cervical cells takes on clear appearance (Fig. 1). A regular repartition of chromatin in small granules is first observed. The coloration intensity diminishes progressively, and DNA shows a fine reticular pattern with perinuclear condensation. The nucleus is then a vacuolated partitioned type.

Cancerous cells exhibit similar changes, but more accentuated (Figs. 1, 2); one may even find a globular fragmentation of the nucleus (Fig. 3). A characteristic feature which is usually found are clear round vacuoles which represent the site of the nucleolus, as shown

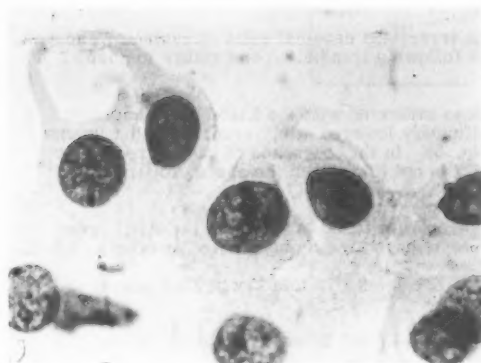


Fig. 1. Benign cervical irradiated cells with increase in nuclear size. Dark dots are not nucleoli but chromatin clumps. (Feulgen reaction-oil immersion.)



Fig. 3. Irradiated cancer cells. (Feulgen reaction - oil immersion.)

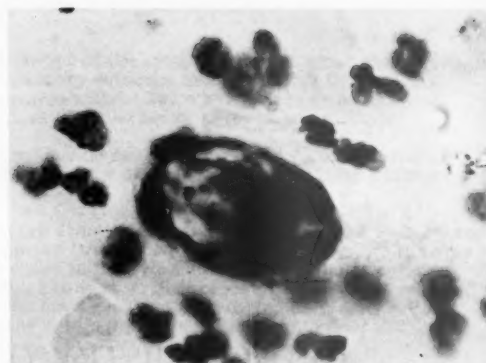


Fig. 2. Irradiated cancer cells. (Feulgen reaction - oil immersion.)

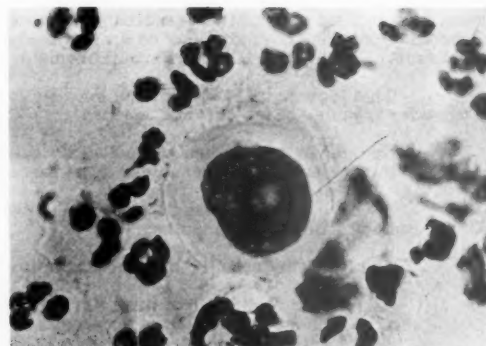


Fig. 4. Irradiated cancer cells. (Feulgen reaction - oil immersion.)

with the Brachet stain. This hole-like appearance in the nucleus is always larger than the nucleolus itself, when stained by the Papanicolaou method (Fig. 4).

2. Increase in the size of the nucleus of benign irradiated cervical cells is evident. The size, however, will change after a period of time following irradiation and return to normal, as shown by nuclear diameters (Fig. 5).
3. The intensity of the Feulgen reaction was also evaluated with the Lison histophotometer. The optical density of cells was found significantly lowered after irradiation, but returned to normal some months after treatment (Fig. 5). In the few cases of cancer recurrence that we studied, we found that optical density is not lowered in the same proportion and maintains higher values (Fig. 6).

In conclusion, an evident diminution of DNA content was found in irradiated benign cervical cells as well as in irradiated cancerous cells. These results have already been noted by others (4, 5, 6).

On the other hand, in cases of precocious recurrence, histophotometry of DNA seems to demonstrate an abnormal response.

This is only, of course, a preliminary report and we cannot as yet evaluate the use of this method in determining prognosis.

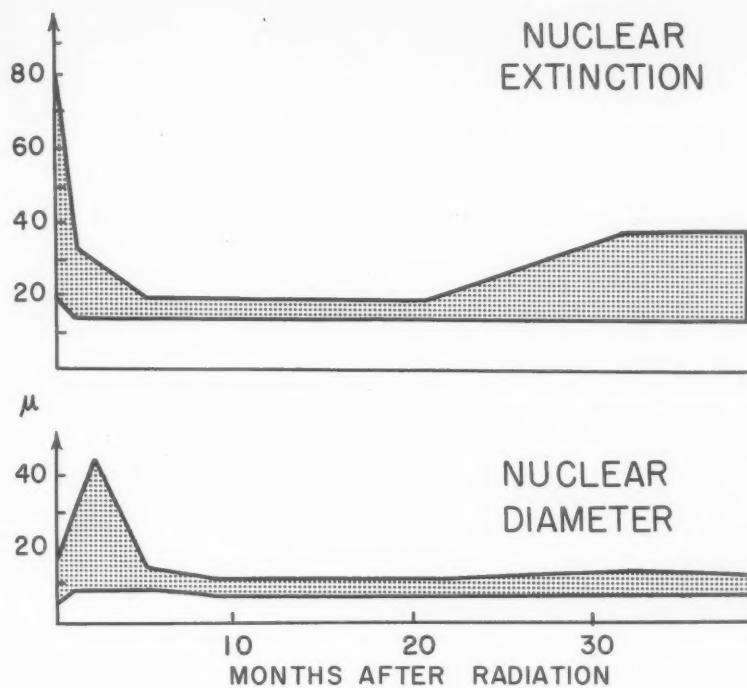


Fig. 5

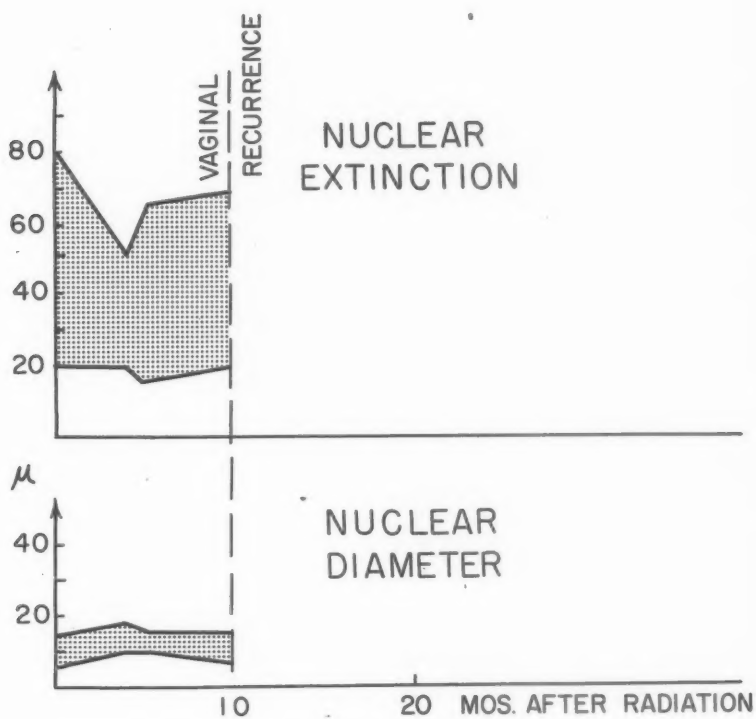


Fig. 6

Bibliography

1. Vokaer, R.: Acta, Symp. on Applied Exp. Cyt., Bruxelles, 1957.
2. Haour, P. and Conti, C.: Acta, Symp. on Applied Exp. Cyt., Bruxelles, 1957.
3. Haour, P. and Conti, C.: Rev. Franc. de Gyn. et d'Obst. 53:No. 9-10, 1958.
4. Caspersson, T. and Sontesson, L.: Acta Radiologica Suppl. 46, 1942.
5. Gusberg, S. B. et al.: Ann. N.Y. Acad. Sc. 63:1447, 1956.
6. Herovici, C.: Acta Cytologica 2:225, 1958.

CONSTANTIN HEROVICI

Villejuif, Seine, France

While we cannot present a complete cytochemical survey of the irradiated cell, we will limit ourselves to the modifications of nucleic acids, glycogen, potassium and alkaline phosphatases, after exposure to ionizing irradiation. These are the cytochemical tests which we use in the daily, routine histological examination.

The material studied is from patients with carcinoma of the uterine cervix and treated at the Institut Gustave Roussy. An initial biopsy and a vaginal smear were taken before treatment. A second biopsy and smear specimens were taken following vaginal application of radium, that is, after dosage of 3.840 mgh to 4.320 mgh had been administered to the uterine cervix. The duration of therapy was twice for three days with an interval of 24 hours. The third specimen was either a surgical specimen, if the therapy after the second biopsy was surgery, or a histological specimen and a cervico-vaginal smear, if the therapy continued to be radiation. The histological specimens were fixed in a mixture of alcohol-formol-acetic acid and the smears were prepared in the usual ether-alcohol fixative.

TECHNIQUE

A. Nucleic Acids

We use two methods for the study of nucleic acids:

The Feulgen technique, which is specific for DNA and is quantitative. The second technique, a combination of methyl-green and pyronine-orange G, shows us the simultaneous equilibrium between the two nucleic acids (DNA and RNA). It also allows us to distinguish between cytoplasm rich (red) or poor (orange) in RNA. Control is done by the ribonuclease test of Brachet.

B. Glycogen

We use Best's carmin red and iodine gum. The advantages of the former is the brilliance of the slides, but the latter is more specific for glycogen. The control is done by salivary amylase digestions to the sections.

C. Alkaline Phosphatase

Our usual technique is the one of Gomori.

D. Potassium

For this mineral we have chosen a modification of the technique of MacCallum by Marza and Chiosa which only takes into account the protein bound potassium.

RESULTS

A. Nucleic Acids

Before the onset of treatment the nuclei of malignant cells of the uterine cervix may be rich or poor in DNA. The quantity of DNA may even vary in different parts of the same tumor. After application of ionizing irradiation, homogenization takes place in the prognostically good cases in the sense of a decrease in the total DNA of the nuclei. The nuclei of the cells from the intermediate and the basal layers take up very little or no methyl green.

The reverse is true with RNA, i.e., it increases both in the nucleoli and in the cytoplasm after treatment. During an experimental study of patients on whom cervico-vaginal smears were taken every two days, we noted that an increase of RNA precedes a decrease of DNA. This observation could be confirmed by studies which Febvre and I performed on epithelial cells in tissue culture which had been irradiated with 1000 r.

In normal irradiated epithelium we could find an increase of the RNA only in the intermediate layer, since this nucleic acid, even without any irradiation, is present in abundance in the basal layer.

The reading and evaluations of the nucleic acids have been carried out with the aid of a histiophotometer.

B. Glycogen

After irradiation one notes large deposits of a substance which stains with Best's carmin. This deposit can be recognized as glycogen since it can be seen in the benign uterine epithelium, which is normally very rich in glycogen. In the second biopsy of the irradiated tumor one can still find these deposits, which in turn detract from the value of this cytochemical test.

C. Alkaline Phosphatase

Phosphomonossterases, localized prior to treatment in the nucleolus and cytoplasm, disappear after irradiation and only in rare cases persist in the nucleoli.

D. Potassium

The intracellular potassium concentration is increased after irradiation, particularly in the cytoplasm. Since this can be observed in the prognostically favorable as well as in the unfavorable cases, this test does not seem to have prognostic value.

We do not attach any prognostic value to these enzyme determinations, since their concentration is often dependent upon hormonal factors. We have been able to induce some variations in their concentrations by a few injections of male hormones.

Thus, as studied in the Institut Gustave Roussy, the cytochemical changes encountered during and after treatment by irradiation with ionizing rays serve as a basis for histoprognois of uterine carcinoma.

DISCUSSION

LUDWIG von BERTALANFFY, Topeka, Kansas, U.S.A. and FELIX D. BERTALANFFY, Winnipeg, Manitoba, Canada:

Observations with the acridine orange (AO) fluorescence method lead to the following results regarding changes of nuclear acids after irradiation:

1. Decrease of DNA was also observed with the AO method (this Symposium).

2. Increase of RNA preceding the decrease of DNA, and found in the cytoplasm as well as in the nucleolus, is indicated by Herovici as a characteristic radiation response. Also with the AO method, a prominent RNA fluorescence, cytoplasmic and nucleolar, was often observed.

It would appear that two stages in the radiation effect can be distinguished: 1) An initial phase with increase in RNA, and 2) after successful irradiation, a subsequent degeneration, with a progressive loss of cytoplasmic RNA (see this Symposium), and with the morphological changes in irradiated cells (see this Symposium).

It is interesting to note that these findings correspond with those on a totally different material, i.e., radiation effects on the cerebellum (1). Thus, Schümmelfeder described also an initial increase in cytoplasmic RNA, particularly with high x-ray doses, followed by rapid necrosis. With smaller doses, a progressive loss of cytoplasmic RNA and slow cell degeneration was observed. Many radiation changes encountered in cervical cells (swelling of cells and nuclei, vacuolization, lumping of chromatin, pyknosis, karyolysis, etc.) were also seen in the cerebellum, indicating that these changes are of a rather unspecific nature.

Bibliography

1. Schümmelfeder, N.: Third Congres International de Neuropathologie 295-308, 1957.

CLOSING REMARKS

PIERRE HAOUR:

In answer to von Bertalanffy and Bertalanffy's discussion, I would like to say that I have had no experience with RNA variation; but I was interested to know that another method, such as the acridine orange fluorescence can also reveal the decrease of DNA content of cells after irradiation.

CONSTANTIN HEROVICI:

I would like to thank von Bertalanffy and Bertalanffy for their judicious remarks. They prove there is a definite prognostic value in recognizing the irradiation reaction, not only in this work, but also in a recent publication on the use of acridine orange under ultraviolet light.

After this publication, we also found an inversion of the fluorescence, which is nuclear before irradiation and mainly cytoplasmic after irradiation.

PHASEMICROSCOPY ON IRRADIATED CELLS

EDMUND SCHÜLLER

Vienna, Austria

Smears were taken from a 47 year old patient, both immediately after treatment with 3600 mgh radium for cervical cancer, Stage III, and ten days after the full dose of 7200 mgh. In the first series, the material was obtained from the cancer-free vaginal wall and from the remaining tumor after cleaning the necrotic films from these areas. The technique of preparation was as described earlier (5). In the second series, after full treatment, the carcinoma had disappeared. These smears contain only benign cells.

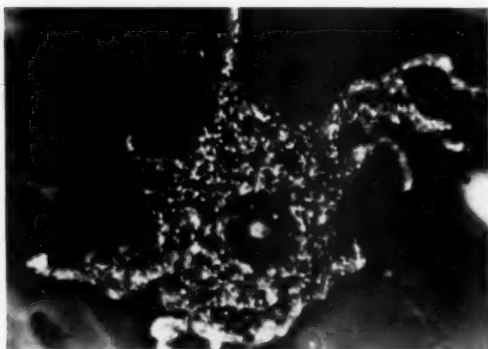


Fig. 1. 3600 mgh radium. Intermediate cell. The cell is increased in size. The nuclear cytoplasmic ratio is preserved. The nucleus is vesicular, its chromatin pattern slightly indistinct. The pseudopodia-like processes of cytoplasm arise at the border of the cell from former vacuoles, which have burst. Anoptal contrast, oil immersion. (All pictures have the same enlargement.)

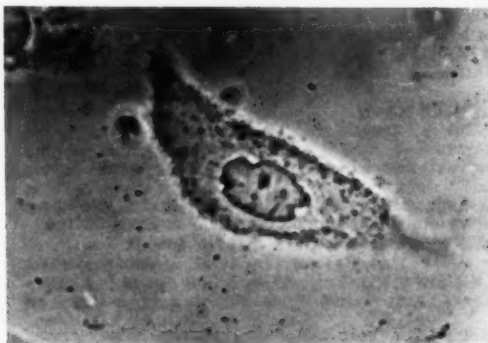


Fig. 2. Basal cell from the same smear as Fig. 1. Cytoplasm and nucleus are almost structureless. The form of the cell is atypical, the enlarged nucleus has a wrinkled membrane. Phase contrast.

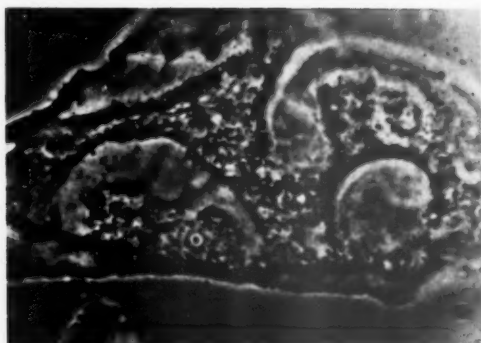


Fig. 3. Intermediate cell (same smear as Fig. 1.). A higher grade of degeneration with vacuoles. The nucleus is more degenerated (right side) than in cells of Figs. 1 and 2. Phase contrast.

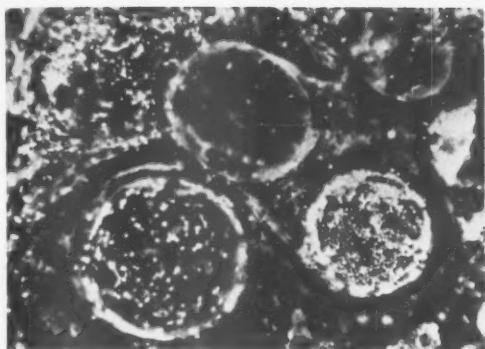


Fig. 4. Smears from carcinomatous areas. Irradiated cancer cells. Large nuclei between indistinct masses of cytoplasm. Two nuclei show a fine granulated karyoplasm or are filled with small drops; one is structureless. Anoptral contrast.

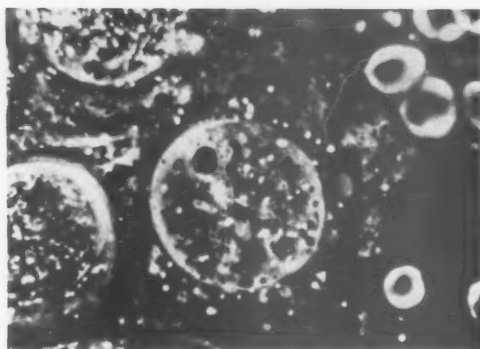


Fig. 5. Another irradiated cancer cell. Bulky crumbs of chromatin seem to lie in a more liquified karyoplasm. Anoptral contrast.

Figs. 1-5 show the immediate response to radiation in the form of more or less well-developed degeneration of cytoplasm and nuclei. If the cellular change is reversible and karyokinesis occurs, then the stimulus of radiation often causes polyploidy (6, 7). Cells of that kind can be found in greater quantities about ten to 14 days after the influence of a high dosage of x-rays or radium. Figs. 6-9 are from smears taken ten days after 7200 mgh radium treatment.

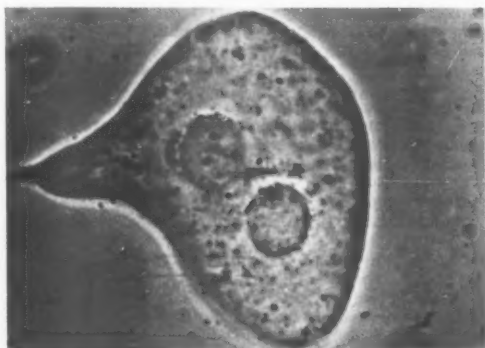


Fig. 6. Enlarged and deformed basal cell with two nuclei. Cytoplasm and nuclei are structureless (usual signs of degeneration after irradiation). The two nuclei are normal in size; therefore, we can assume that it is a tetraploid cell with two diploid nuclei in the interphase. Phae contrast.

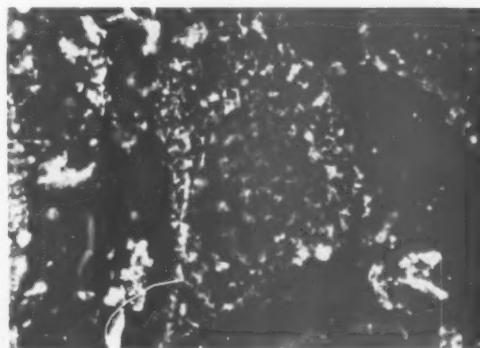
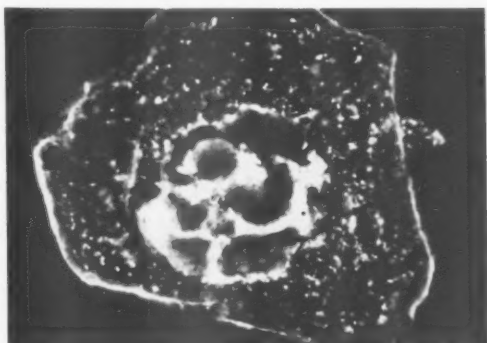


Fig. 7. A degenerated squamous epithelium in combination with a gigantic nucleus after high dosage of radium. The nuclear-cytoplasm ratio is disarranged. Both cytoplasm and nucleus show signs of degeneration. In this case the enlargement of the nucleus is explainable by polyploidy. The chromosomes of a cell with two diploid nuclei have, during karyokinesis, united into one large nucleus. Benign irradiated cell with three nuclei, one small, two greater and equal in size have been shown (5). The cause of this phenomenon is the same. Anoptral contrast.

Fig. 8. Multilobulation of a large nucleus in a highly differentiated intermediate cell. The cytoplasm shows almost no signs of degeneration. The nucleus has formed into a great mulberry-like mass. This is also an effect of polyploidy. In this case the irradiation prevented a complete division of chromosomal masses in a single nucleus. Anoptral contrast.

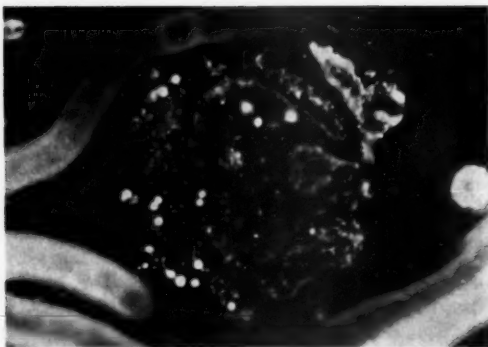


Fig. 9. A squamous epithelial cell with a large nucleus and signs of general degeneration. The nuclear cytoplasm ratio is disarranged. Such abnormally large nucleoli in a gigantic nucleus can be found in cases of polyploidy, when the chromosomes double without earlier division, Anoptal contrast.

The morphological changes after cellular irradiation consist of all grades of degeneration of cytoplasm and nuclei, and of polyploidy in the next cell generation.

Bibliography

1. Politzer, A.: Wien klin. Wschr. 63:629, 1951.
2. Politzer, A. and Albertini, G.: Ztschr. Zell. u. Gewebsl. 1:413, 1924.
3. Schüller, E.: Strahlentherapie 89:456, 1952.
4. Schüller, E.: Zbl. Gyn. 57:409, 1953.
5. Schüller, E.: Acta Cytolog. 2:315, 1958.
6. Winkler, H.: Ztschr. Botanik 8:417, 1916.
7. Undritz, E.: Hämatologische Tafeln Sandoz. Basel, 1952, Sandoz A. G.

ULTRAVIOLET ABSORPTION IN SQUAMOUS EPITHELIAL CELLS EXHIBITING RADIATION RESPONSE

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One of the marked changes to occur in squamous epithelial cells during radiotherapy is an increase in size of both nucleus and cytoplasm. This increase in cell size has been interpreted as evidence of hydropic degeneration. It was postulated that the permeability of the cell membrane was changed by irradiation and for that reason more water entered the cell. The result was a ballooning of both cytoplasm and nucleus. It should be possible to determine if this is so by ultraviolet microscopy. If the cytoplasmic constituents are diluted by any great amount of water the cytoplasmic absorption of these large epithelial cells should be less than in the perfectly normal epithelial cell.

One hundred and eighteen cells from six patients without carcinoma and without radiotherapy were examined in the ultraviolet microscope for the amount of absorption in normal cells. One hundred and sixty-four cells were examined from ten patients with carcinoma of the cervix treated by radiotherapy, all of whom had a good cytologic response to radiation. The vaginal secretion for ultraviolet microscopy was taken somewhere near the peak of the patient's response, in order to have cells exhibiting marked radiation response. The instrument used was the Polaroid Color Translating Ultraviolet Microscope. Those interested in the details of this microscope are referred to the paper mentioned below (1).

The cells examined under ultraviolet light have been divided according to three quantities of cytoplasmic absorption - slight, moderate and marked. The difference between slight and moderate is in the intensity of the cytoplasmic absorption as judged by definite color differences. Marked absorption refers to those cells whose cytoplasmic absorption was so intense that an increase in exposure time was required for any cellular detail to be seen in the ultraviolet light.

Normal benign squamous cells and benign squamous cells showing radiation effect have a rather marked difference in the ultraviolet absorption of their cytoplasm. No differences in nuclear absorption have been seen. Thirteen cells or 11% of the normal cells showed moderate absorption at the wave lengths 280, 261 and 240 μ . The remaining cells have only a slight absorption. No cell was encountered with marked absorption at these wave lengths. By contrast, in the benign cells showing radiation effect, 55 cells or 33% showed moderate absorption at 280, 261 and 240 μ . Thirteen cells or 8% showed marked absorption requiring at least four times the normal exposure before any cellular detail could be seen. These highly absorbent cells were seen in three of the patients (1).

Not only do the benign radiated cells differ in the quantity of ultraviolet absorption, but they also differ in the quality. No normal benign cell was encountered with any cytoplasmic absorption above 280 $m\mu$. In the cells showing radiation effect, 28 or 17% showed cytoplasmic absorption at 289 $m\mu$, and sixteen or 10% had some cytoplasmic absorption at 297 $m\mu$.

It may be of passing interest to note that the vacuoles encountered in radiated cells show no absorption in the ultraviolet light. These vacuoles have been examined in a number of cells from wave lengths as low as 235 $m\mu$ to as high as 365 $m\mu$. No absorption was encountered at any wave length. It is probable that they contain only water and electrolytes.

This increase in both the quantity and the quality of absorption of ultraviolet light could be explained by an actual increase in the large protein molecules in the cytoplasm. Rather than suggesting that these large radiated cells are evidence of hydropic degeneration, it, on the contrary, suggests that there is some kind of temporary growth stimulation in these cells, in which the cytoplasmic constituents are actually increased in amounts. Some of these constituents may be compounds which are different than those found in the normal cell, since the quality of ultraviolet absorption is quite different in the cells showing radiation effect.

Many of the discussants in this symposium have indicated that they consider the cells showing radiation effect as degenerate. I think that there is little cytological evidence for such a supposition. The cells showing radiation effect do not stain lighter than normal cells but often more intensely. Their nuclei are not degenerate but well preserved. The cytoplasm absorbs much more intensely in the ultraviolet light. This would indicate that we are dealing with a rapid stimulation of growth in the cytoplasm of these benign cells rather than with degeneration. Attempts should be made to distinguish the two phenomena.

Bibliography

1. Graham, R. M. and Crozier, Ruth: Annals of the N. Y. Acad. of Sciences 63:1202, 1956.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

FLUORESCENCE MICROSCOPY OF IRRADIATED CELLS

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Experience on radiation cell changes with fluorescence microscopy is still limited. The following statements can be made at present.

The advantage of the fluorescence microscopic method is in the fact that it permits one, by use of an easy and rapid technique, to follow cytochemical changes in malignant cells, including those occurring under radiation. The morphologic changes induced by radiation and discussed elsewhere in this symposium can easily be ascertained with this technique. Additionally, the method under consideration reveals cytochemical characteristics both of proliferating and of degenerating malignant cells as follows:

The acridine orange fluorescence method introduced by the author (1-5) is essentially based upon the differentiation, with the fluorochrome acridine orange, of the two kinds of nucleic acids of the cell. DNA of the nucleus appears in green, and RNA of the cytoplasm in brown to orange to red fluorescence. Proliferating malignant cells generally have a high content of cytoplasmic RNA and therefore are conspicuous by their brilliant cytoplasmic fluorescence, even with low magnification. Degenerating cells, on the other hand, progressively lose their cytoplasmic RNA and hence their flaming fluorescence.

This particularly applies to the effects of radiation. It appears that irradiation especially attacks the nucleic acids of the cell and hence those cytochemical components with direct protein synthesis and cell growth, thus leading to progressive degeneration and eventually necrosis of malignant elements. This can be seen in smears examined with the fluorescence method.

Nuclei of irradiated cells often show depletion of DNA so that only some green lumps or traces are seen in the sometimes "ballooned" nuclei; in stages which appear to be of advanced degeneration, the vesicular nuclei often are optically empty. Cytoplasmic RNA appears to show an initial increase (5, 6) and hence particularly strong, red fluorescence of the cytoplasm. Otherwise many malignant cells show marked reduction and vacuolation of the cytoplasm, loss of cytoplasmic RNA and consequently of the brilliant red fluorescence. Cells in what appear to be ultimate stages of degeneration, show nuclei, often ballooned and bizarre, with faint green fluorescence or optically empty, naked or surrounded by a narrow rim of cytoplasm depleted or empty of RNA and hence appearing in faint green fluorescence. Debris from decomposed cells, appearing reddish, is often a characteristic feature of radiation smears.

These cytochemical changes which are easily observable with the acridine orange fluorescence method, in particular the gradual loss of cytoplasmic RNA, may be a valuable diagnostic criterion which expresses the success of radiation. The rapidity and simplicity of the method allows for cytologic evaluation on the spot and therefore would recommend it for quick check-up during radiation treatment. Detailed follow-up studies of patients undergoing radiation therapy are presently under way (7).

Bibliography

1. von Bertalanffy, L., & Bickis, I.: *J. Histochem. Cytochem.* 4:481-493, 1956.
2. von Bertalanffy, L., Masin, M., & Masin, F.: *Cancer* 11:873-887, 1958.
3. von Bertalanffy, L., & Bertalanffy, F. D.: *What's New (Abbott) No. 214 (Early Winter):* 7-9, 1959.
4. von Bertalanffy, L., & Bertalanffy, F. D.: *Morphology. Discussion. This Symposium of ACTA CYTOLOGICA.*
5. von Bertalanffy, L., & Bertalanffy, F. D.: *Cytochemistry of irradiated cells. Discussion. This Symposium of ACTA CYTOLOGICA.*
6. Herovici, D.: *Cytochemistry of irradiated cells. This Symposium of ACTA CYTOLOGICA.*
7. Bertalanffy, F. D., & Goodwin, A. M.: *Unpublished results.*

COLPOMICROSCOPY OF THE IRRADIATED UTERINE CERVIX

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Our observations of patients with cervical carcinoma treated by x-ray are confined to a few limited cases, which were treated intra-vaginally according to the "Guttinger method." These patients all had progressive cervical carcinoma of Stages III and IV. Because of necrosis and hemorrhage, the surface of such progressive carcinoma has been changed before radiation to such an extent that it is often difficult to find unchanged carcinoma tissue on the surface, when using the colpomicroscope. However, all cases were examined colpomicroscopically as far as possible during the process of radiation. After an approximate dose of 1500 r, the first changes of the carcinoma cells could be proven. The staining properties of such cells deteriorated rapidly. The size of nuclei increased and the chromatin structure became indistinct. Frequently changes of the nuclei were found corresponding to karyorrhexis. The cytoplasm, which scarcely can be found in untreated carcinoma because of the very close aggregations of cells, frequently showed vacuolization. At a dose of about 2500 to 3000 r this vacuolization of cells increased to such an extent that cell borders appeared only as a network without nuclei or the nuclei appeared to be loose in the cellular network. After termination of the radiation treatment (mostly 6000 to 7000 r intra-vaginally) it was practically impossible to prove the existence of carcinoma cells on the surface, because of the considerable increase of necrosis.

A prognosis in respect to radiation sensitivity or resistance could not be deduced from the colpomicroscopical findings in our few cases. It also must be considered that even the manipulations in the course of the intra-vaginal radiation can inflict mechanical damage to the delicate carcinoma tissue, which can lead to false conclusions.

Summarizing, it was demonstrated that in all of our cases during the course of radiation, a rather uniform increase in the vacuolization of the cytoplasm occurred. In the nuclei concerned, an enlargement occurred and also a decrease in the chromatin structure, resulting in karyorrhexis.

COLPOSCOPY OF THE IRRADIATED UTERINE CERVIX

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Graz, Austria

Macroscopically we find the irradiated cervix shows a very similar picture to that observed in older women. Such findings can also be colposcopically verified to some extent. In addition, however, it is possible by means of colposcopy to recognize certain changes as being the result of irradiation and thus to distinguish them from the physiological changes of senility.

All of these changes effect the mucous membrane of the whole vagina. It is, therefore, also possible to recognize them even if the cervix cannot be made visible, as a result of the rather frequent conglutination of the vagina following irradiation.

The most striking feature is the atrophy of the mucous membrane. It appears thin and to some degree transparent. Capillaries, which are rather frequent in older women, are but sporadic in irradiated cases. Accordingly, the mucous membrane looks pale white or yellowish-white in color. The few vessels that may be observed usually take a straight course, with occasional coarse meshed nets of vessels which cross one another. Minor lesions of the surface (*erosia vera artificialis*) are rather frequent, and they arise as a consequence of atrophy of the mucous membrane. These lesions rarely produce bleeding when irradiated. Occasionally, however, very small hematomas can be observed beneath the epithelium.

Just as in senility, the ectocervix is always covered by squamous epithelium after irradiation. Ectopies of the irradiated cervix could not be observed in the investigated cases.

The immediate effect of irradiation upon a tumor of the cervix is characterized by the development of a typical colliquation necrosis, later by the development of large ulcerative lesions which heal slowly and finally, by the new formation of the cervix. In this respect colposcopy is confined to a somewhat more accurate differentiation of the stages which are already recognizable macroscopically.

Finally, early recurrences of irradiated carcinomas show the same colposcopic changes as the primary early stages of carcinoma of the cervix.

WARREN R. LANG

Philadelphia, Pennsylvania, U.S.A.

Although the colposcopic technique has been only infrequently applied to the study of the cervix after radiation therapy, the method does offer an additional aid in evaluating irradiation changes of the epithelium, in addition to serving as an effective means for the detection and localization of local recurrence of carcinoma.

The colposcopic findings of early and late cervical cancer need only be briefly mentioned. Early changes include the various forms of leukoplakia (simple, mosaic and ground), an abnormal transformation zone with a glassy appearance and irregular blood vessels or perhaps only areas of non-iodine staining squamous epithelium. In more advanced lesions the above characteristics are supplemented by evident ulceration and proliferation. None of these changes, however, is in itself specific for cancer.

With radiation therapy the above findings disappear after several weeks. If proliferative - ulcerative changes were originally present, and if the malignancy responds well to radiation, the lesion slowly shrinks and, following a destruction of cancerous tissue, the cervical area heals in with a covering epithelium. As this is happening and until healing is complete, a radiation epithelitis with hyperemia is present.

Assuming that the cancer is brought under control, and varying with the intensity and mode of radiation, the cervix itself may or may not maintain the form of a cervix. Usually the vaginal vault contracts and the cervical site becomes smooth, possibly with a small nubbin present. Under such circumstances, colposcopy, often somewhat difficult technically because of contracture, reveals characteristic surface patterns. The epithelium appears thin, taking the iodine stain poorly. No glandular epithelium is present. Irregular surface vascularization is apparent, but the vessels, unlike those of malignancy, are frequently large and lacking in corkscrew or comma patterns. Areas of fibrosis manifested as colposcopic leukoplakia are frequent. If too much radiation is administered, necrosis occurs and a dirty sloughing crater ensues.

Local recurrence, in our opinion, can be suspected with the aid of colposcopy, and we have done so on two occasions when there were no suggestive clinical findings. In both cases the smear was suspicious. With colposcopic guidance we were able to locate the site of abnormality and therefore find the site of malignancy.

ARNALDO de MORAES AND JOÃO PAULO RIEPER

Rio de Janeiro, Brazil

In order to make feasible an evaluation of the colposcopic aspect of the irradiated cervix, it is necessary to compare it to the picture presented by the carcinomatous tissue before the radiation therapy.

Vascularization is one of the more characteristic elements of the carcinoma in its early period and in its more advanced phases of growth.

The study of the blood vessels belongs particularly to colposcopy, since it visualizes them in their functional condition and can evaluate their distribution, thickness, course, and state of repletion in an easy and ideal fashion which is not possible by histological examination of material obtained by biopsy or surgical specimens.

This particular advantage of colposcopy should be stressed and employed in the diagnosis of cervical cancer as well as in the study of the irradiated cervix.

In the carcinomatous tissue one observes capillary loops, corkscrew or hair-pin shaped. As compared to the vascularization of other cervical lesions where the vessels spread in a single plane and have regular ramifications, in carcinomatous tissue the vessels show up as being isolated, without apparent connections, with variable thickness, and often disappearing abruptly in a pointed fashion. The abundant and irregular distribution of the vascular elements in an irregular grayish or yellowish tinged tissue is very characteristic of carcinoma of the cervix.

What change appears in these pictures under the influence of radium or x-rays?

We have practically never seen a total reverse of the vascular pattern in clinically cured cases, as has been described by Rieck, who found aspects similar to those of senile colpitis.

In our experience there always remain irregular vessels in the mucosa or irradiated cervixes, even years after clinical cure. These are short vessels, with an irregular course, without apparent connections, with variable lumina and disorderly distribution, and in an atrophic grayish mucosa. Beside these vessels, other vascular nets are also found with ramifications such as are usually seen in sequelae of cervicitis.

These irregular vessels of irradiated cervixes are not identical to those of growing carcinomatous tissue, but sometimes resemble them.

From our point of view, it is not possible to determine the cure of cervical carcinoma after irradiation through colposcopy alone. This method offers some evidence which may be helpful, but it can never be conclusive.

DISCUSSION

ERNST H. KRÜGER, Halle a.d. Saale, Germany:

I agree with Bajardi, Lang and Moraes and Rieper. However, regarding the colposcopic picture, I would like to add the following:

A. Findings after intra-uterine radium insertion for carcinoma of the uterine corpus.

Thinned epithelium and a transparent atrophy-like vascular pattern with numerous filiform, long, stretched, superficial and vulnerable vessels are found. The external os shrinks and takes a crater-like aspect, and therefore, the visible grape-like structures on the squamo-columnar junction disappear.

B. Findings after intracervical radium insertion for:

1. Endophytic-growing carcinoma of the uterine cervix.

The markedly noticeable vascular patterns, which almost always can be observed, disappear during treatment.

2. Exophytic-growing carcinoma of the uterine cervix.

There is no complete normalization. However, improvement of the characteristic vascular pattern of the exophyte occurs with intact surface.

Corkscrew and spider web-like vessels disappear, and the remaining ones are long, stretched, very superficial and vulnerable. They are similar but not the same as the ones which can be observed with colpitis on senile-atrophic transformation zones. However, colpitic foci are absent, while numerous subepithelial bleeding spots are seen. The previously reddish-yellowish, shiny appearing epithelium of the uterine cervix becomes glazy and level after irradiation, as a consequence of the thinning of the epithelium.

The degeneration of insufficient atypical vessels with cervical carcinoma after irradiation cannot safely be used as a test of the radiosensitivity of the tumor and therefore does not allow a prognostic evaluation of the case.

Beginning local recurrences are easily and early appreciable by the colposcope. However, the differential diagnosis between a radiation ulcer and a carcinoma ulcer colposcopically cannot always be made with certainty.

OTAKAR NYKLÍČEK, Náchod, Czechoslovakia:

The introduction of a vaginal speculum causes the greatest technical inconvenience during the colposcopic examination of the cervix after irradiation. This is very painful for the woman and very often bleeding occurs.

We had the opportunity of observing (immediately after the radium had been removed) colposcopic changes on the cervix, determined by Hinselmann as leukoplakia, mosaic, abnormal transformation zone or ground. We directed our efforts mainly on investigating the changes of the capillaries, which are very important for the colposcopic diagnosis of cervical cancer. Observations could be undertaken immediately after the cessation of radiation, later on conglutination of the vagina occurs.

First, changes could be observed on the blood vessels. The sufficient ones developed into insufficient ones for a short while, bleeding heavily from contact and forming subepithelial hematomas. This occurred to capillaries that were situated nearest to the radium applicator. Later this process ceased and the vessels lost their "wild" picture, nevertheless they were visible for a long time in the atrophic mucosa. The mosaic, as well as the ground, quickly disappeared and was gradually replaced by a thin epithelium. The immediate effect of irradiation of a tumor of the cervix, such as colligation necrosis and the slow healing process of the defect, have been observed equally according to the description of the main speakers.

We further agree that colposcopy proves to be a very efficient investigation method for detecting early recurrences of irradiation carcinomas.

CLOSING REMARKS

WARREN R. LANG:

Krüger's listing of the differences of radiation-induced colposcopic changes with intra-uterine radium insertion for carcinoma of the uterine corpus and those for cervical carcinoma are well taken. The changes, however, do overlap, not only because of different methods of radiation techniques, but also because of variations of host response, location of the carcinoma and the reactivity of the cancer itself. It is certainly true that radiation and carcinomatous ulcerations may be difficult to distinguish colposcopically. But then cytologic as well as histologic changes of radiation and carcinoma may also be confusing. Colposcopic examination of the lower genital tract after irradiation may be difficult as stated by Nyklíček. However, correctly sized and shaped specula, patience, and experience easily overcome this minor handicap.

HORMONES AND RADIO SENSITIVITY OF THE VAGINAL EPITHELIUM OF RATS

LOUIS J. DARCIS

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- I. **INTRODUCTION:** Considering that "radiation response" is of prognostic significance in the treatment of cervical cancer (8, 16, 17, 18, 19, 20, 27, 28, 30, 31), it becomes important to study the factors which alter it.

Elevation of the oxygen concentration (1, 6, 7, 15, 24, 26, 29), temperature (2, 5) or cellular metabolism (with thyroline for instance) (4, 25, 32) increases the radiosensitivity of different tissues but is not very practical.

Hormones profoundly modify the radiosensitivity of animals irradiated in toto (3), and J. B. Graham (23) and J. B. and R. M. Graham (21, 22) improved the radiation response of patients with cervical cancer by administration of testosterone.

We hope to have experimentally proven, in a series of works which have been described in detail elsewhere (9, 10, 11, 12, 13) and which will be briefly summarized here, that hormonal factors can change the radiosensitivity of a tissue (in this particular instance the vaginal mucosa of rats) toward local irradiation.

- II. **MATERIAL, TECHNIQUE AND METHODS:** All animals (albino rats weighing from 150 to 175 gm.) were irradiated by means of a tube of radium (50 mgh) introduced into the vagina and left in place two or four hours, according to the experiment. General anesthesia was maintained during the entire duration of irradiation in order to avoid extrusion of the tube.

Every day a vaginal smear of each animal was taken, stained with the usual Papanicolaou technique and studied as to the percentage of radiated cells it contained.

III. RESULTS

- A. We define as an irradiated cell every cell which contains at least two nuclei or the largest diameter of which is greater than 45 μ . Not more than 10% of such cells are present in a normal vaginal smear (Fig. 1-4).
- B. The percentage of irradiated cells varies with the time elapsed after exposure to radiation. After a latent period ranging from three to five days, irradiated cells appear between the fourth and sixth day; their frequency reaches a peak between the sixth and tenth day and then progressively decreases to reach normal values again between the eighth and fifteenth day, sometimes later.
- C. For the same duration of radiation, the highest percentage of irradiated cells ranges within relatively narrow limits. On the other hand, it varies as a function of the duration of irradiation:
1. After four hours of irradiation the highest percentage of irradiated cells fluctuates between 45% and 70%, with an average of $60.4 \pm 3.4\%$.
 2. After two hours of irradiation the highest percentage of irradiated cells fluctuates between 23% and 36% with an average of $29.2 \pm 1\%$.

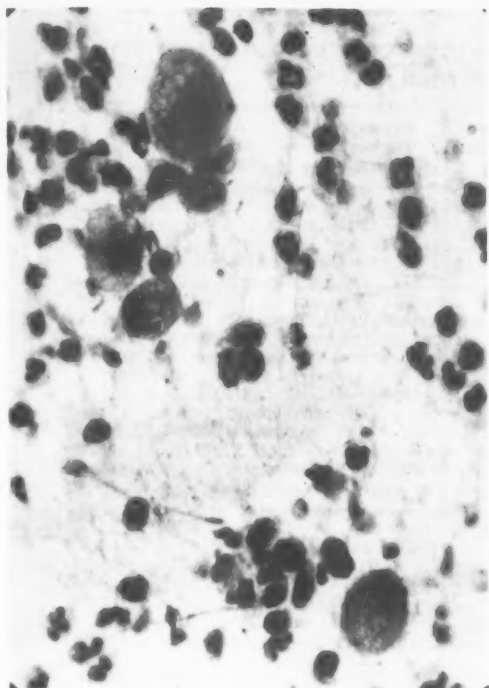


Fig. 1

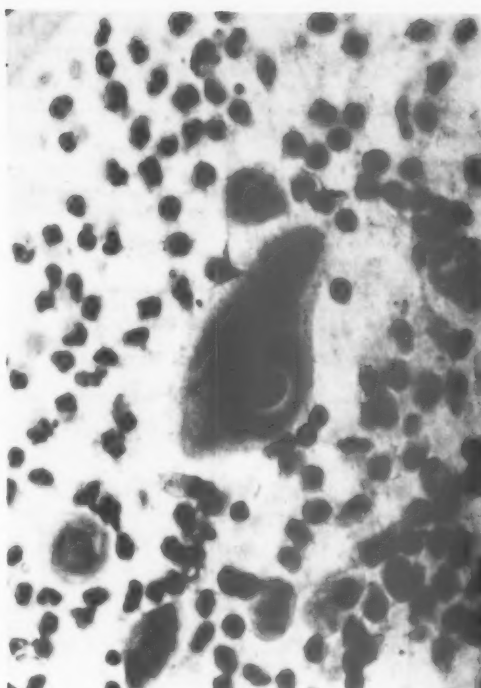


Fig. 2

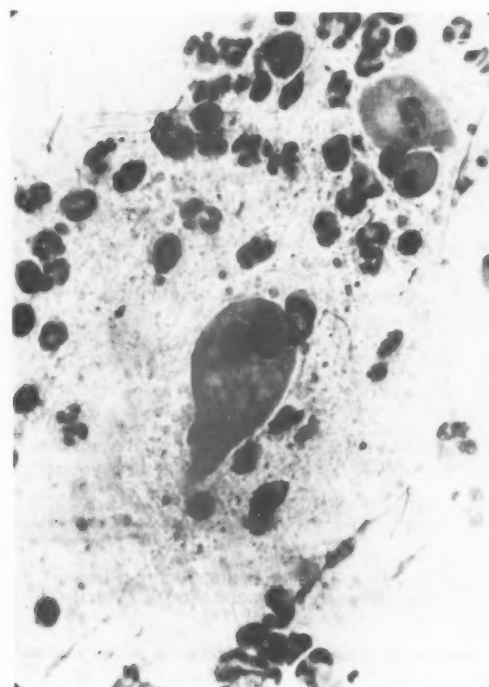


Fig. 3

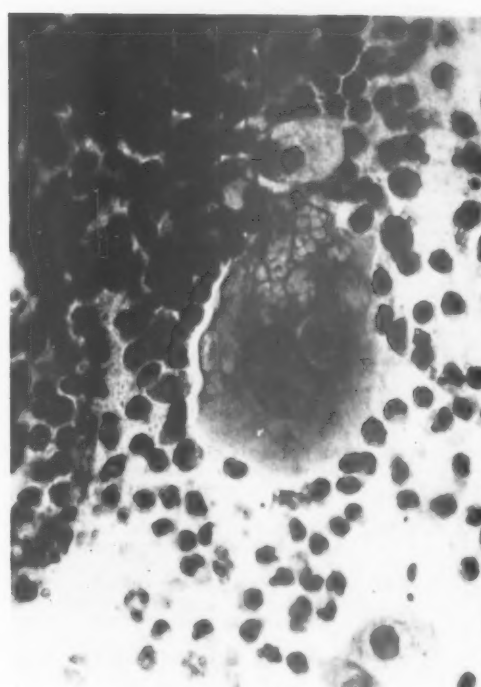


Fig. 4

TABLE I.
INFLUENCE OF DIFFERENT ENDOCRINE FACTORS ON THE RADIO-SENSITIVITY
OF THE VAGINAL EPITHELIUM

	Treatment	No. of Animals	Average of Highest %; Radium 2 hrs.	Average of Highest %; Radium 4 hrs.
Irradiation Alone	None	18	29.2 (\pm 1)	
		21		60.4 (\pm 3.4)
Gonads	Testosterone	11	48.5 (\pm 1)	
	Estradiol Benz.	11	36.7 (\pm 1.6)	
	Progesterone	11	16.7 (\pm 0.7)	
	Castration	10		38.5 (\pm 1.6)
	Cast. & Test.	$\left\{ \begin{array}{l} 5 \\ 7 \end{array} \right.$	32.6 (\pm 0.8)	49.5 (\pm 1.6)
Adrenals	Cortisone	11	29.1 (\pm 4.2)	
	D O C A	9	36.6 (\pm 1.1)	
	Adrenalectomy	9	35 (\pm 1.3)	
Pituitary	Chorionic Gonado- Trophin	$\left\{ \begin{array}{l} 7 \\ 5 \end{array} \right.$	40.9 (\pm 3.2)	54.4 (\pm 3.6)
	Somatotrophic Hormone	9	35.9 (\pm 3.2)	
	ACTH	8	20.3 (\pm 0.9)	
Thyroid	Thyroxine	9	49.1 (\pm 1.2)	
	Radio-Thyroidectomy (I_{131})	$\left\{ \begin{array}{l} 11 \\ 5 \end{array} \right.$	34.7 (\pm 1.1) 32.6 (\pm 1.4)	
	Surg. Thyroidectomy	11	31.3 (\pm 2.8)	
Pregnancy	None	10		35.4 (\pm 1.4)

D. The influence of different endocrine systems on the radio-sensitivity of the vaginal mucosa has been investigated. Table 1 summarizes our findings.

It can be seen that:

1. Estradiol and chiefly testosterone increase the radiation response of the vaginal mucosa, while castration and progesterone decrease it.
2. The adrenals play essentially no role in conditioning the sensitivity of the vaginal mucosa toward local irradiation. This is marked contradiction to the essential role they play toward total body irradiation.
3. Thyroxine increases the radiation response while radio-thyroidectomy with I_{131} , as well as surgical thyroidectomy are without effect.
4. ACTH decreases the radiation response while chorionic gonadotrophin, and to a lesser degree somatotrophic hormone, increase it. It is interesting to point out that chorionic gonadotrophin increases the radiosensitivity of the ovaries as well (14).
5. Pregnancy seems to confer an important resistance toward local irradiation in the vaginal mucosa of rats.

- IV. COMMENT: The possibility of modifying by biological means the radiosensitivity of a given tissue might represent an important step in the radiological treatment of malignant tumors. In relation to this problem, we have experimentally demonstrated that different hormonal influences are able to modify, sometimes to a considerable extent, the sensitivity of the vaginal mucosa of rats to local irradiation.

Bibliography

1. Anderson, R. and Turkowitz, H.: *Am. J. Roentg.* 46:537, 1941.
2. Bacq, Z. and Alexander, P.: *Principes de Radio Biologie.* Liege, 1955, Sciences et Lettres.
3. Betz, E.H.: Contribution a l'etude du syndrome endocrinien provogue pas l'irradiation totale de l'organisme. Liege, 1955, G. Thone.
4. Blout, H.C. and Smith, W.W.: *Science* 109:83, 1949.
5. Clarke, J.: *Am. J. Roentg.* 40:501, 1938.
6. Conger, A.D.: *Radiology* 66:63, 1956.
7. Crabtree, H.G. and Cramer, W.: *Proc. Roy. Soc. Biol.* 113:238, 1933.
8. Cuyler, W.K.: *Proceedings of Symposium of Exfoliative Cytology* Oct. 23-24, 1951, New York, 1953, American Cancer Society Inc.
9. Darcis, L.: Influence des glandes endocrines sur la sensibilité de la muqueuse vaginale de la rate aux radiations (Derouaux), Liege, 1956.
10. Darcis, L., Onkelinx, C. and Hotterbeex, P.: *Ann. Endocrinol.* 17:300, 1956.
11. Darcis, L. and Gilson, G.: *Experientia* 13:242, 1957.
12. Darcis, L. and Hotterbeex, P.: *Experientia* 14:18, 1958.
13. Darcis, L.: *Bull. Soc. Roy. Belge Gyn. et Obst.* 27:97, 1958.
14. Desai, P.: *Acta Radiol.* 41:545, 1954.
15. Evans, J.C., Slaughter, C., Little, P. and Failla, G.: *Radiology* 39:663, 1942.
16. Graham, R.M.: *Surg. Gyn. and Obst.* 84:153, 1947.
17. Graham, R.M.: *Surg. Gyn. and Obst.* 84:166, 1947.
18. Graham, R.M.: *Surg. Gyn. and Obst.* 93:767, 1951.
19. Graham, R.M. and Graham, J.B.: *Cancer* 6:215, 1953.
20. Graham, R.M. and Graham, J.B.: *Cancer* 8:59, 1955.
21. Graham, J.B. and Graham, R.M.: *Cancer* 6:68, 1953.
22. Graham, J.B. and Graham, R.M.: *Ca., A Bulletin of Cancer Progress* 5:56, 1955.
23. Graham, J.B.: *The Laboratory Diagnosis of Cancer of the Cervix (Homburger and Fishman).* Basel, 1956, S. Karger.
24. Gray and Coll.: *Brit. J. Radiol.* 26:638, 1953.
25. Haley, T.J., Mann, S. and Doudy, A.H.: *Endocrinology* 48:365, 1951.
26. Hultborn, K.A. and Forssberg, A.: *Acta Radiol.* 42:475, 1954.
27. Kjellgren, O.: *Acta Radiol. Suppl.* 168:1958.
28. Kohn, G.: *Acta Radiol.* 41:446, 1954.
29. Mottrom, J.C.: *Brit. J. Radiol.* 8:643, 1935.
30. Nielson, A.M.: *Acta Radiol.* 37:479, 1952.
31. Shier, C.B.: *Am. J. Obst. and Gynec.* 67:286, 1954.
32. Smith, W.W. and Smith, F.: *Am. J. Physiol.* 165:651, 1951.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
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THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

RADIATION CELL CHANGES IN EXPERIMENTALLY PRODUCED CARCINOMA OF THE CERVIX

EMMERICH von HAAM AND RICHARD ALBERY

Columbus, Ohio, U.S.A.

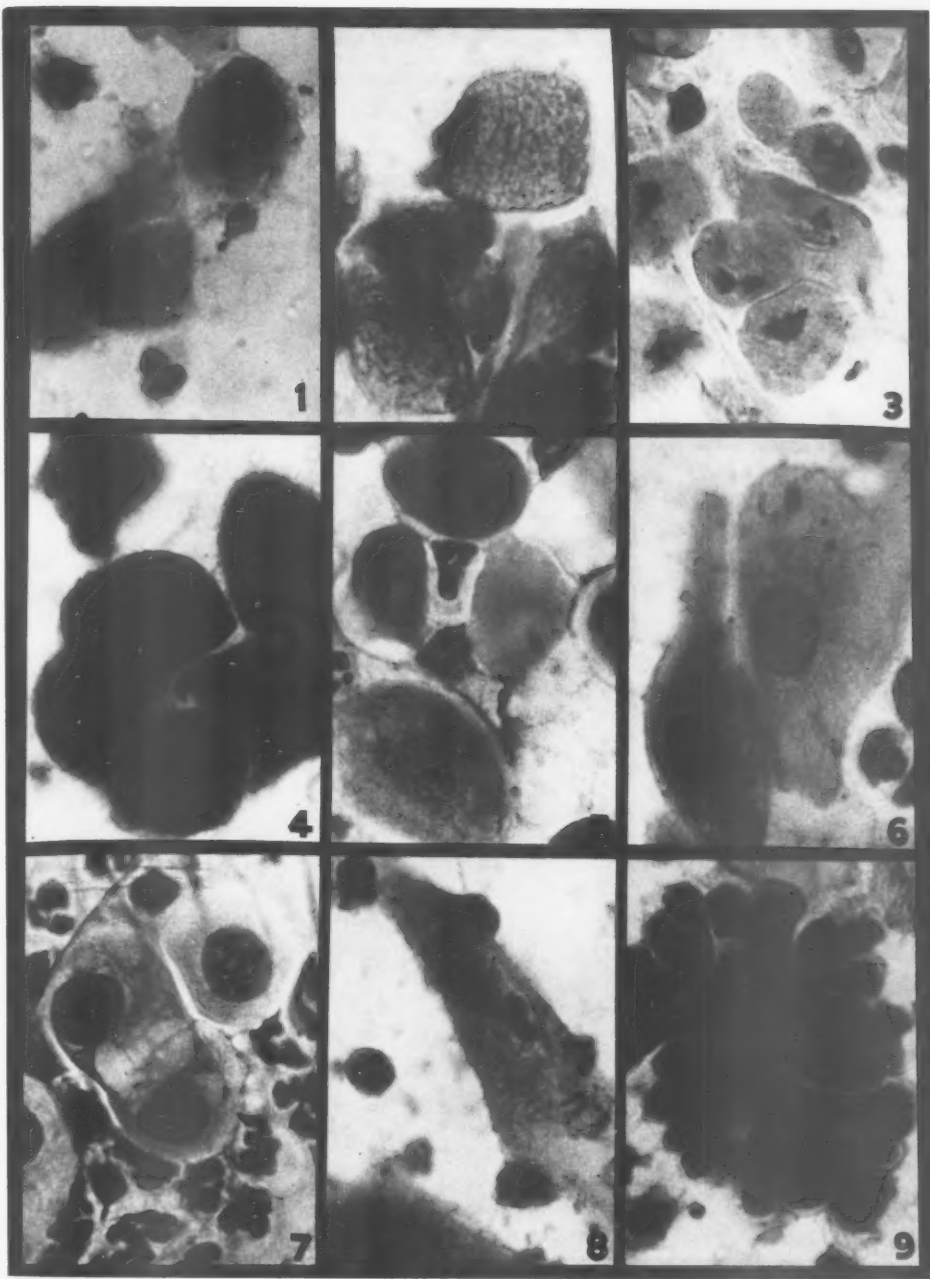
The following discussion is based upon a series of experiments during which mice in various stages of tumor development were subjected to external radiation of 400 r delivered, by a 45 kilowatt machine, alternately to the front and back portions of the pelvic region at weekly intervals. The remaining portions of the animals were shielded carefully against radiation effect. This was accomplished by placing the animal in an out-stretched position in a narrow cage covered with 1/8 inch thickness of lead, which permitted exposure of the desired regions only. The animals were anesthetized with ether during the procedure. After each radiation treatment the animals appeared quite ill; they developed diarrhea and sat motionless in the corner of the cage. Vaginal smears were taken the third day following the treatment. A few normal mice died during the treatment, while the mortality among mice with tumors was rather high. Each animal received a total of 2400 r during a period of six weeks.

Results: Radiation changes in exfoliated cells appeared in the third week of the experiment and persisted from then on. In normal control animals the parabasal cells enlarged and showed coarse or fine vacuoles in the cytoplasm. The nucleus also enlarged but the chromatin pattern remained unchanged (Fig. 1, 7). In the intermediate cells mucification was an outstanding feature and in many animals superficial or cornified cells were completely replaced by these mucified cells (Fig. 2). This was interpreted as evidence of a decrease in the estrogenic hormone by the effect of the radiation upon the ovaries, as described by Rugh and Wolff (1). It represents the vaginal response to subthreshold doses of ovarian hormones as postulated by Fluhmann (2). The superficial cells, whenever they persisted, showed marked nuclear changes with karyorrhexis and karyolysis (Fig. 3). These changes were always accompanied by a short-lasting leukocytic reaction, and leukocytes appeared to collect around clumps of necrotic cells in the manner described by von Haam (3) in vaginal smears of human cases subjected to radiation therapy (Fig. 9).

In animals which showed cellular dysplasia as a result of the administered carcinogen, the parabasal cells assumed an appearance not unlike those described by Graham (4) as "radiation response." The cytoplasm became dense and intensely basophilic. The cell volume as well as the size of the nucleus increased, but the nuclear-cytoplasmic ratio remained unchanged (Fig. 4). Mucification effect was not observed in dysplastic cells; instead, they increased enormously in size and showed paranuclear vacuoles, cytoplasmic granules and large nuclei with prominent chromatin (Figs. 5, 6). It was felt that the difference between normal and dysplastic cells in our animals was definitely greatly enhanced by radiation therapy. Karyolysis and karyorrhexis observed in these large, atypical superficial cells produced grotesque pictures of cytoplasm with bits of nuclear material scattered throughout (Fig. 8). In many cells two pyknotic nuclei were present as a result of amitotic division.

Changes in malignant cells were prominent and occurred usually earlier than in normal or dysplastic cells. The undifferentiated small malignant cells (Fig. 10) enlarged and showed heavy vacuolization, which often pushed the nuclei to a marginal position (Figs. 13, 16). The more differentiated malignant cells (Fig. 11) assumed grotesque figures with marked pyknosis in the large nuclei (Fig. 14). The pearly bodies found in the vaginal smears of animals with well-differentiated tumors (Fig. 12) became swollen and vacuolated (Fig. 16). Amitotic division appeared quite frequently (Fig. 18) and karyorrhexis and karyolysis were also observed (Fig. 17).

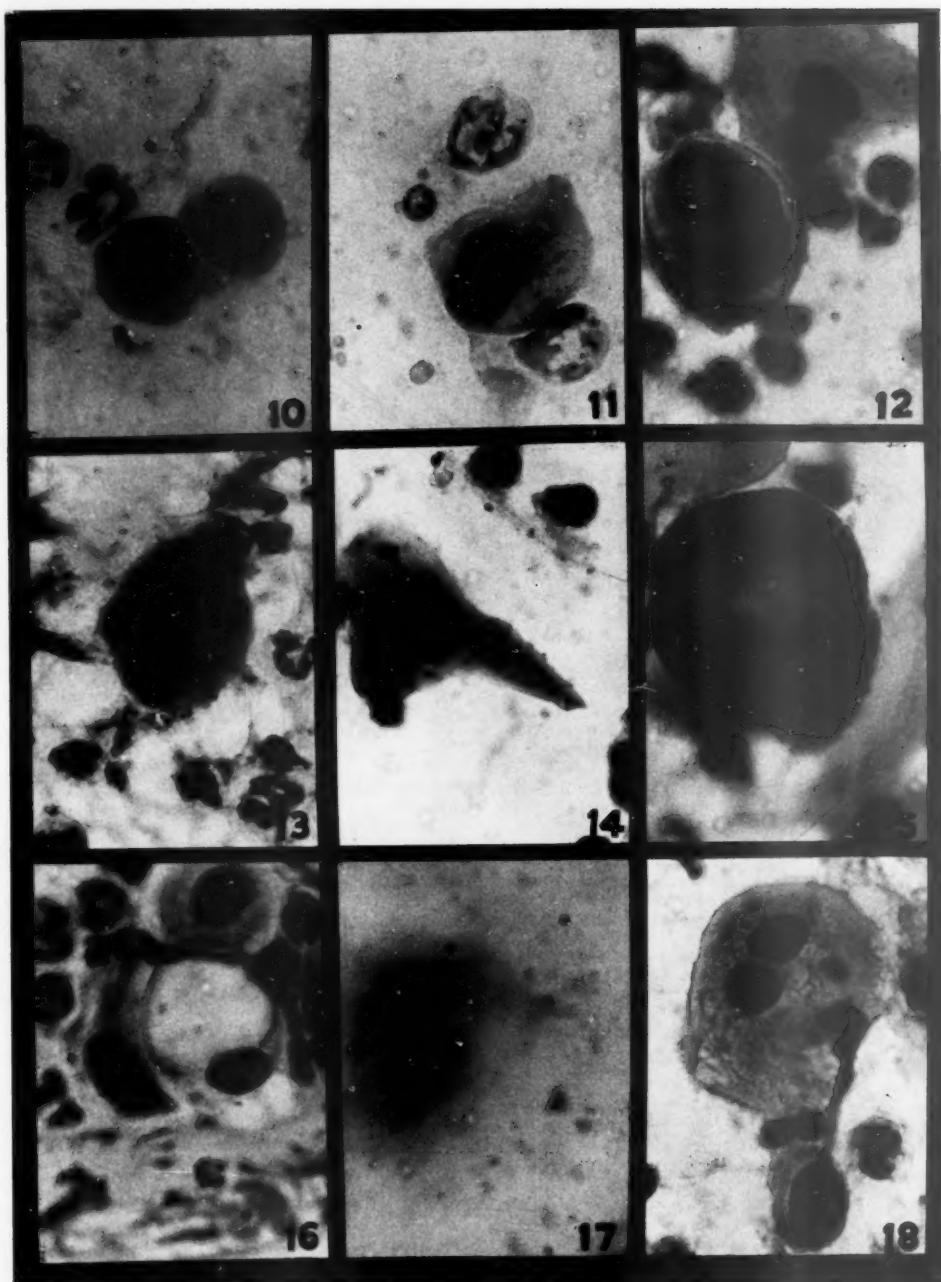
These cellular changes were accompanied by profuse leukocyte response with hemorrhage. In animals with advanced tumors no clumping of leukocytes was observed, and white blood cells mingled diffusely with the tumor cells. Since the estrous cycle had ceased to exist in animals with advanced tumors, no hormonal changes were noticeable following radiation.



Radiation Changes in Normal and Dysplastic Cells (Oil immersion, X1200)

- Fig. 1. Irradiated, normal parabasal cells
 Fig. 2. Irradiated, intermediate cells showing mucification
 Fig. 3. Irradiated, superficial cells
 Fig. 4. Irradiated, atypical basal cells
 Fig. 5. Irradiated, dysplastic intermediate cells

- Fig. 6. Irradiated, dysplastic superficial cells
 Fig. 7. Irradiated, normal parabasal cells showing vacuolization
 Fig. 8. Irradiated, dysplastic superficial cells showing karyorrhexis
 Fig. 9. Clumps of leukocytes around necrotic material



Radiation Changes in Malignant Cells (Oil immersion, X1200)

- Fig. 10. Undifferentiated malignant cells
 Fig. 11. Differentiated malignant cells
 Fig. 12. Pearly body from differentiated carcinoma
 Fig. 13. Irradiated undifferentiated cell
 Fig. 14. Irradiated differentiated cell

- Fig. 15. Pearly body after irradiation
 Fig. 16. Vacuolization in irradiated malignant cells
 Fig. 17. Karyolysis in irradiated malignant cell
 Fig. 18. Amitosis in irradiated malignant cell

In summary then, in experimental animals we could observe radiation changes which closely resembled those described for the human being. The changes involved the cytoplasm as well as the nuclei and produced pictures markedly different from the exfoliated cells of unirradiated animals. The study of chronic radiation changes as well as the effect of radiation upon the histological appearance of tumors will be discussed in a later publication.

Bibliography

1. Rugh, R. and Wolff, J.: Fertil. & Steril. 7:546, 1956.
2. Fluhmann, C. F.: Am. J. Physiol. 95:422, 1930.
3. Von Haam, E.: Am. J. Clin. Path. 24:652, 1954.
4. Graham, R. M.: Surg. Gyn. & Obst. 84:153, 1947.

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MINIMAL DOSAGES OF X-RAYS OR MINIMAL DOSAGES OF RADIUM WHICH PRODUCE RADIATION CELL CHANGES

EDMUND SCHÜLLER

Vienna, Austria

For this study intravaginal x-ray irradiation is very well suited. With intravaginal x-ray therapy using the 60 KV hollow anode tube, a rather heavy dose must be given to the surface, i. e., vaginal epithelium, in order to obtain the required amount of radiation in the deep tissues. Fortunately, the resistance of the vaginal wall to x-rays is many times that of the skin and other mucous membranes.

I have found, after a minimal dose of one irradiation of 980 r on the surface, the first evident signs of cellular radiation response in a low percentage of all desquamated cells. The changes - vacuolization of the cytoplasm, increase in size of the whole cell, and definite nuclear changes - can be seen about three days after treatment. The peak of cellular changes is found 10 to 14 days after irradiation. At this time you can also find single cells with multiple nuclei.

Immediately after a lower dose (500 r) and with daily controls during a period of two weeks, the only response to radiation is in the form of a greater mass of unchanged desquamated cells.

DISCUSSION

OLLE KJELLGREN, Gothenburg, Sweden:

Are the radiation changes in the vaginal smear dependent on the given radium dose or are there significant host factors which are of importance for the intensity of the radiation reaction? This is a question of immense and central importance in the study of radiation reaction and its prognostic significance. I have been studying this problem from different points of view and have analyzed the relationship between the radiation reaction in the vaginal smear and the given vaginal radium dose. The investigation showed that within therapeutic limits the radiation reaction might be either poor or good, independent of whether the radium dose was low or high. The doses were, however, measured in mgh, which is not quite an adequate way of estimating the radium dose. At present we are analyzing the correlation between the intensity of the radiation reaction in the vaginal smear and the dose measured in r in the rectum and the bladder. The investigation is, however, not yet completed.

Bibliography

1. Kjellgren, O.: Acta Radiol. Suppl. 168, 1958.

NO CLOSING REMARKS

THE HISTOLOGICAL CRITERIA OF RADIATION RESPONSE

ALFRED GLÜCKSMANN AND CORA P. CHERRY

Cambridge, England, U.K.

After treatment of patients with radiocurable tumors, a fibrous scar is the only indication of the previous existence of a carcinoma, while in patients with radio-incurable cancers the active tumor tissue persists. Depending on time and dose of radiation treatment and on the response of the carcinoma, intermediate stages between these two extremes are found. Radiation acts directly on tumor cells, indirectly via the blood vessels and the stroma of the tumor, and, in addition, systemic factors modify the reaction to irradiation of both normal and malignant structures.

In any given tumor the cell population varies in its constituents with the developmental stage of the focus. In old parts degenerating and differentiating, i. e., keratinizing or secretory cells predominate, while in young foci the mitotic and the resting cells, i. e. the stock cells of the tumor, are the most numerous. Direct, indirect and systemic actions of radiation influence the composition of the tumor population, and for purposes of comparison the youngest tumor foci should be analyzed.

The effects of radiation on non-viable, i. e., differentiated and degenerating cells, cannot be assessed accurately and are of relatively little importance. The viable cells, i. e., the resting and dividing cells, have great potentialities for further growth, and the effect of radiation on these types of cells is of the greatest importance. Effects of radiation on these viable cells are: (1) inhibition of division and damage to the reproductive apparatus which leads subsequently to abnormal mitosis and possibly cell death; (2) lethal effects, i. e., immediate break down of cells by various processes of degeneration; (3) increase in extent and degree of differentiation, i. e., more cells keratinize or secrete and reach a higher degree of keratinization; (4) increase in cell and nuclear size resulting in multi- or mononucleated monster cells. Parallel changes occur in the stroma producing an often excessive fibrous and cellular proliferation with breaking up of tumor foci and scattering of cells. These effects are predominantly evidence of radiation injury to the cancer, but are not incompatible with subsequent recovery of the tumor from persisting unchanged viable cells. Thus, in any tumor biopsy taken during irradiation, both the injurious effects of radiation and the superimposed attempts at regeneration of both normal and malignant tissues are found.

The influence of systemic factors on radiation response is seen in premenopausal patients with carcinoma of the uterine cervix, in whom the reaction of the normal epithelium is correlated with that of the tumor, while in postmenopausal patients the response of the normal epithelium does not vary with the local radiocurability of the cancer.

Bibliography

1. Glücksmann, A.: Recent advances in clinical pathology. London, 1947, J. & A. Churchill Ltd.
2. Glücksmann, A.: Acta, Union Internationale Contre le Cancer 14:358, 1958.
3. Cherry, C. P. and Glücksmann, A.: Cancer 7:504, 1954.

DISCUSSION

HANS F. BETTINGER, Melbourne, Australia:

I quite agree with the authors' description of the radiation changes but the argument in the last paragraph of their contribution is too condensed to follow. Would the authors please amplify.

S. B. GUSBERG and GRACE HERMAN, New York, New York, U.S.A.:

Our program of Radiosensitivity Testing of cervical carcinoma is, in its histological aspects, comparable to this one. Our assessment is based on the following changes after a test dose of irradiation: cell and/or nuclear enlargement, chromatin pattern alterations, nuclear and/or cytoplasmic vacuolization, multinucleation, increase in size and number of nucleoli, degree of increased differentiation, and stromal response. In the final RST designation, stress is placed on the amount of minimally altered tumor remaining.

There are differences in our testing method worth noting:

1. We utilize one, or at times two, test doses of irradiation. In this way a dose large enough to produce a spectrum of response is delivered, yet the radiation is not great enough to interfere with surgical treatment if such a mode is dictated by the poor response. The standardized test dose has resulted in a range of tumor response that has allowed prognostication of healing with a 70-75% accuracy. In Stage I and II lesions 3000 r is delivered via trans-vaginal cone.
2. We have found it possible to assess radiation change in differentiated cells.
3. The mitotic index or change in mitotic index has not proved helpful prognostically.
4. Our technique includes wherever possible nuclear cytological and cytochemical scanning.
5. We take our samples from the fresh but not the growing edge of the tumor.

EMMERICH von HAAM, Columbus, Ohio, U.S.A.:

Dr. Glücksmann's concise description of the histological criteria of the radiation response must be commended. I wonder if in his observation the "youngest" tumor foci are always found in the periphery of the tumor, or if such foci may also occur in the form of islands within growing tumor tissue?

WOLFGANG KORTE, Bonn, Germany:

One should remember that we do not know specific tissue reactions which are only characteristic for the influence of radium, x-ray and radioactive cobalt on human tissue. Cells and tissues in proliferative and regressive processes obey fixed laws. The same morphological phenotypes may be induced by different damages or influences.

As is well known, spontaneous necrosis in quickly growing malignant neoplasms is not rare. In such foci or necrosis, but also in their vicinity, changes of nuclei and of cytoplasm can be found without influence of radiation, which the experienced individual also, is not able to differentiate from changes induced by radiation, if no additional items of information are available. The chronically proliferative, as well as the purulent inflammation in the neighborhood of a neoplasm is able to produce a similar picture.

If one knows the growth picture in a neoplasm before the beginning of the radiation treatment, one is justly allowed to attribute changes in this picture during or after irradiation to the radiological influence.

In the main I agree with the opinions of Glücksmann and Cora Cherry. However, I do not think that the described changes in the connective tissue and vessel walls are produced by the effect of radiation. The connective tissue group (active mesenchyme) at once responds to every degeneration of the neighboring, higher differentiated cell complex. For this there are numerous examples in pathology, which are not related to blastomas or radiation effects.

I want to complete the paper of Glücksmann and Cora Cherry by adding some photographs from my own material. If one intends to proceed methodically, one should distinguish, according to Leibach, radiologically intensified changes in the nuclei (and nucleoli) as well as in the protoplasm.

I. Nuclear Changes

1. Nuclei swelling: (Fig. 1)
2. Disturbances of mitoses: (Fig. 2)
3. Clumping of chromatin: (Fig. 3)
4. Vacuoles of nuclei: (Fig. 4)
5. Formation of giant nuclei, formation of giant cells: (Figs. 5 and 5a)
6. Swelling and degeneration of nucleoli: (Fig. 6).

II. Cytoplasmic Changes

1. Vacuolization: (Fig. 7)
2. Liquefaction: (Fig. 8)
3. Eosinophilic homogenization: (Fig. 9)
4. Cornification: (Fig. 10).

The following photographs were prepared from material from the Department of Obstetrics and Gynecology, University of Bonn (Direktor: Prof. Dr. H. Siebke). All examined tissues have been excised, excised and extirpated during the patient's lifetime. Fixation in formol (one part concentrated solution (40%) and 6-9 parts water). Embedding in paraffin. Gauge of sections between 4-12 microns.

I. CHANGES OF NUCLEI

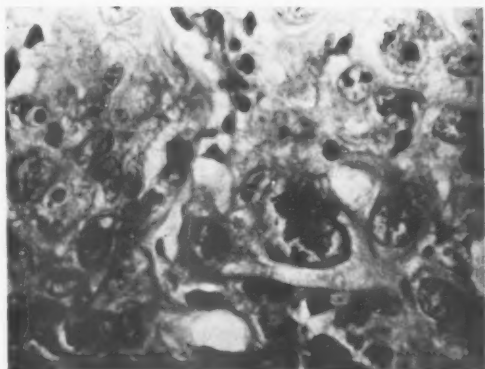


Fig. 1. Enlargement of nuclei

General enlargement of nuclei, so-called "nuclear swelling." H1073/55 excochleation. Neoplasm of colli uteri. Cornifying squamous cell carcinoma. Eight days after the last of two insertions of radium, altogether 4000 mgh. Magnification 10x45.

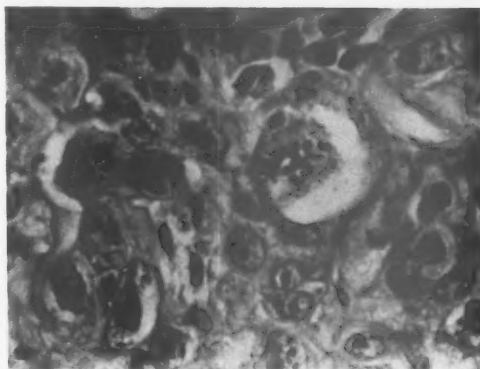


Fig. 2. Disturbance of mitoses

Two typical crumbly-like mitoses. H-209/55 excochleation. Neoplasm of colli uteri. Exophyte. Squamous cell carcinoma with polymorphic cells. 4000 mgh of radium, 3000 r surface dosage. Six days after last radium insertion, 10x45.



Fig. 3. Clumps of chromatin

Bizarre forms of nuclei. H-1110/55 Wertheim operation. 15 days after total 4000 mgh of radium. Compare with legend No. 1. 8x45.

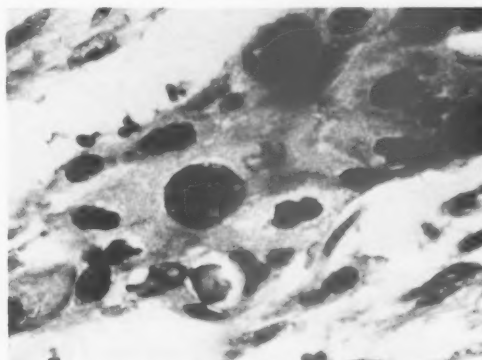


Fig. 4. Vacuoles of nuclei

Fine vacuolization of giant nuclei H 565/57 excochleation. Neoplasm of colli uteri. Giant exophyte. Not cornifying squamous cell carcinoma. Seven days after the last of two insertions of radium. Altogether 4000 mgh 10x45.

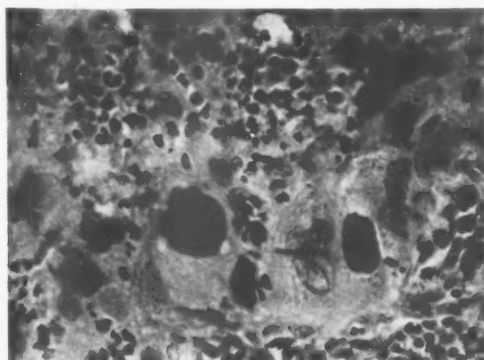


Fig. 5. Giant nuclei

Grotesque giant nuclei. Numerous inflammatory cells H-352/58 Wertheim operation. Neoplasm of colli uteri. Broken up deep knot. Not cornifying squamous cell carcinoma. 15 days after 2500 r radioactive cobalt. 8x45.

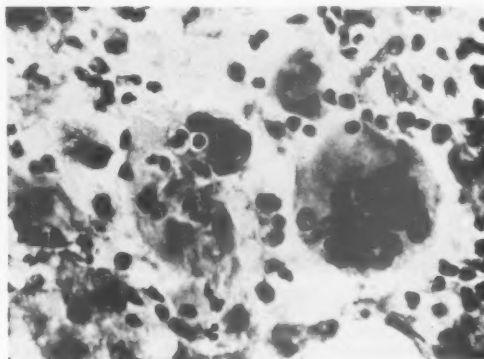


Fig. 5a. Polynucleate giant cells

Originated from phagocytosis, amitosis or apposition. H 1110/55 Wertheim operation. Compare with legend No. 3. 10x45.

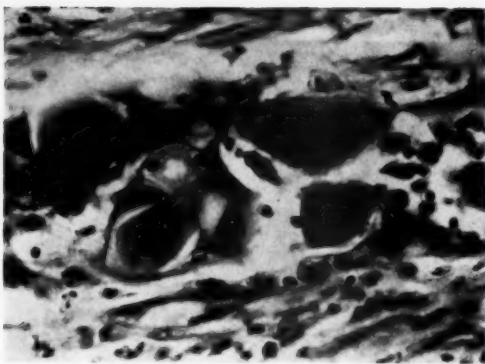


Fig. 6. Degeneration of nucleoli

Rhexis of nucleoli, especially in the small cell under the giant nucleus. H 1110/55 Wertheim operation. Compare with legends Nos. 3 and 5a. 8x45.

II. CHANGES OF CYTOPLASM

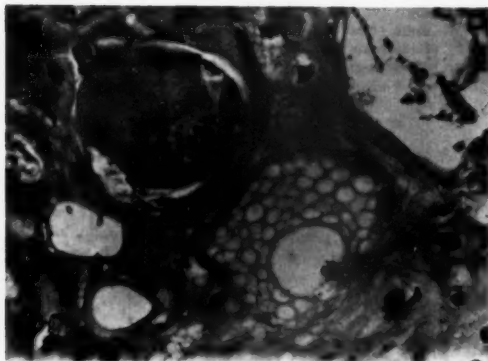


Fig. 7. Vacuolization

Small and large blistered formation of vacuoles in the protoplasm. H1034/55 excochleation, Neoplasm colli uteri. Broken-up deep knot. Cornifying squamous cell carcinoma. After 4000 mgh of radium, eight days after 40 days of pendular convergence irradiation. 8x45.



Fig. 8. Liquefaction

Liquefaction and vacuoles of the protoplasm. H111/57 excochleation, Neoplasm of vagina. Not cornifying squamous cell carcinoma. Eight days after three insertions of cobalt (altogether 10,000 r) 8x45.



Fig. 9. So-called eosinophilic homogenization

Note especially the protoplasm at the bottom, on the right. Vacuoles of protoplasm. H111/57 excochleation. Legend as in topic II, Fig. 2. 8x45.



Fig. 10. Cornification

Cornification with symptoms of degeneration of the nuclei and the protoplasm. H1034/55 excochleation. Compare legend Topic II, Fig. 1. After radium and pendular convergence irradiation. Eight days after the last convergence irradiation. 8x45.

Staining according to Weigert - van Gieson or with hematoxylin-eosin. Photographs with the Leitz-Panphot. Periplan optic. Automatic arc light. Silver eosin photographs 9x12 cm.

Bibliography

1. Leibach, W.K.: Grundlagen und Praxis der Bewegungsbestrahlung, Volume 2, Wuppertal-Elberfeld, 1955, W. Birardet.

CLAUD W. TAYLOR, Birmingham, England, U.K.:

Observations made over many years by Glücksmann and colleagues from the Strangeways Laboratory have given a basis for histological assessment of the response of tumors to irradiation. Sensitivity to irradiation depends to a large extent on inducement of the tumor cells to differentiate. Foci of viable carcinoma are converted into non-viable cells and ultimately to masses of keratinized material often surrounded by foreign-body giant cells. Indirect tissue reaction replaces the destroyed carcinomas by fibrous connective tissue.

A varying picture is seen according to the nature of the carcinoma, tissue response, dose of irradiation and the time interval between therapy and histological examination. The most difficult problem for the pathologist is to distinguish between viable and non-viable tumor cells in some foci of residual carcinoma. Grossly degenerate and well differentiated cells are distinct from typical viable cells but minimal damage may mimic or obscure cell differentiation. Can some of the tumor cells recover from the damage they have received?

The possibility of residual foci of viable carcinoma being rendered non-viable by limitation of blood supply due to later indirect tissue reaction also has to be considered.

ANTOINE ZAJDELA and MICHEL COTON, Paris, France:

We do not concur with the opinion expressed by the authors of the paper, according to which x-rays and radium possess a power to increase differentiation in malignant tumors, which already have some kind of differentiation.

We believe that in a malignant tumor (for instance squamous-cell or secretory-cell carcinoma) the undifferentiated cells, in a comparatively great number, degenerate and are absorbed faster than the more differentiated cells (parakeratotic, cornified or secretory cells) after injury caused by irradiation. The appearance of increased differentiation (parakeratotic, cornified or secreting) of the tumor, during or following irradiation, results from this. An example is in certain sterilized squamous cell carcinomas where everything may have been absorbed except for masses of keratinized squames, often surrounded by reactive giant cells.

Moreover, the radiation dose does not seem to have any effect on the process of differentiation of the malignant tumor cells.

We would also emphasize that the various histological changes of certain irradiated malignant tumor cells (enlargement of the cell, multi- or mono-nucleate giant cells, deeper staining, cytoplasmic vacuolization) cannot be interpreted as a differentiation process, but only describe the cell being injured by radiation.

It has been noted, on the other hand, that in any characteristic type of tumor (for instance the squamous cell carcinoma), the radiosensitivity is most pronounced when the rate of anaplastic cells is highest. A well vascularized stroma is also a good prognostic omen.

Andersen's histological studies support our view that therapeutic radiation of a number of theoretically radiosensitive tumors is not followed by histological proof of a differentiation process.

In our opinion, present day knowledge in morphology does not seem to justify the use of histological response in a malignant tumor during radiation as a means of foretelling the clinical response of the tumor to radiation.

Bibliography

1. Andersen, S.: Investigations into Differentiation and Other Morphological Changes in Malignant Tumors Following Therapeutic Irradiation With X-Rays and Radium. Copenhagen, 1949.
2. Lacassagne, A. and Gricourof, G.: Action des radiations ionisantes sur l'organisme. Paris, 1956,

CLOSING REMARKS

ALFRED GLÜCKSMANN and CORA P. CHERRY:

Dr. Taylor has raised the problem of the degree of cellular damage compatible with recovery. For tumors, this implies the ability of the cell to reproduce indefinitely under favorable environmental

conditions. Experimental evidence suggests that apparently undamaged irradiated cells may accomplish a few divisions but ultimately become sterile, while others though appearing cytologically damaged may recover and resume their reproductive activity. It is impossible to give a histological or cytological definition of minimal, though ultimately fatal, radiation injury applicable to all tumor cells, particularly since environmental conditions influence the process of recovery. For practical purposes we find that cells of squamous cell carcinomas of the cervix and the oral cavity may be considered as viable if they show no morphological change or when, in spite of some morphological alterations (increase in volume, etc.), they are able to enter prophase and/or retain a foamy cyanophilic cytoplasm. Swelling or shrinkage of the nucleus with accompanying changes in the chromatin pattern, condensation and increased eosinophilia of the cytoplasm can be taken as an indication of permanently impaired viability.

We agree with Taylor that environmental conditions can control the activity of residual tumor foci. The 'recurrence' of tumors in secondary sites many years after treatment illustrates this phenomenon. The nature of the local and systemic (?immunological, ?endocrine) factors responsible for the control of growth of these residual tumors is not known, though their use in therapy might be of great importance. Unfortunately, we find that in a series of well over 3,000 patients such control was effective for five or more years in only about 6% of cases with radioresistant carcinomas.

Dr. Bettinger asked us to amplify the statement in the last paragraph: In an investigation of the radiation effects on the normal cervical epithelium of patients with radiocurable and with resistant carcinomas we found that the height of the epithelium and the number of cells per unit volume of the epithelium decreased progressively in premenopausal patients with radiocurable tumors, but only transiently in premenopausal patients with refractory tumors. In post-menopausal patients the decrease in height of the epithelium and in the number of cells was the same for patients with radiocurable and with refractory tumors. These observations confirm the basic finding of the Grahams that systemic factors influence alike the reaction to irradiation of normal and malignant tissues. The difference between pre- and post-menopausal patients suggests that endocrine conditions may play a role in this control and the persistence of a high cornification count in patients with refractory tumors points in the same direction.

We fully endorse the statement by Gusberg and Herman about the prognostic importance of the persistence of minimally altered tumor tissue. We agree that variations in the mitotic index shortly after exposure are of limited prognostic significance, but find that a high mitotic index observed long after a heavy dose of radiation is an unfavorable prognostic sign.

The chances of finding the most reactive tumor foci are greatest at the growing edge, though they occur also in other parts of the tumor as von Haam suggests, provided necrotic and ulcerated regions are avoided. Drs. Gusberg and Grace Herman seem to obtain adequate material near but not at the growing edge.

Histochemical tests (Gusberg, Herovici, v. Bertalanffy) promise to be of great use in finding out why some tumors respond to irradiation and other fail to do so. As a result of such investigations they may in future increase the prognostic accuracy of histological and cytological examinations.

We agree with Korte on all but two points: we deplore the use of Formol as a routine fixative since the cytological results are unsatisfactory and erratic and we prefer, for cytological detail, fixation with Susa, Zenkeracetic or Bouin. The second point of difference is the statement that the mesenchymal (? and vascular) reaction of the proximal tumor bed is secondary to degenerative changes in the tumor parenchyma. Undoubtedly the tumor tissue influences the tumor bed, but there is equally good evidence (1) that the tumor bed affects the tumor (see Taylor's remarks above) and (2) for direct radiation effects on the blood vessels. The progressive endarteritis obliterans follows radiation so frequently and rapidly as to become almost typical for radiation injury though it occurs also under other pathological conditions.

The points made by Zajdela and Coton can be answered as follows:

1. The relative increase in the proportion of differentiating to undifferentiated cells is due not only to the disappearance (by degeneration and resorption) of the latter but also by their becoming differentiated and keratinized. This can be shown in normal and malignant tissues by using micronuclei and binucleate formations as markers and thus tracing the progress of cells from the basal layers. Cells in these layers undergoing division during or after irradiation produce, as a result of injury, these abnormalities and can be followed from the basal layer to the surface or to the center of tumor foci during their process of keratinization. Thus, keratinized cells seen seven or more days after exposure can be demonstrated to have been undifferentiated cells at the time of exposure.
2. In radiocurable tumors there is also an increase in the degree of differentiation and "better" keratin is formed (i. e., more uniform eosinophilic and picophilic condensation of the cytoplasm with loss of nuclei instead of mere parakeratotic changes). The increase in the amount of secretion per cell (as well as of the numbers of cells) in irradiated tumors reveals the often unsuspected columnar component in the mixed carcinomas of the uterine cervix.
3. Increased differentiation has been described in clinical material (Dominici & Barcat, 1907; Hamperl & Schwarz, 1927; Hall & Friedman, 1948) experimental tumors (Friedman & Drutz, 1958) and in normal tissues (Friedman et al., 1955; Cherry, 1957). Even Andersen describes definite relative and absolute increases in the cornification of the mouse epidermis. His work on clinical material gave a confused picture because he failed to distinguish between radiocurable and refractory cancers. His main experimental work was done on a "comparatively radioresistant" tumor which though derived from a squamous cell carcinoma had lost its capacity for differentiation during a series of transplantations.

4. The frequently repeated dogma that anaplastic tumors are radiosensitive (and by implication locally radiocurable) lacks supporting evidence. There is a close correlation between the incidence of differentiated squamous cell carcinomas and the cure rates at ten accessible sites (Glücksmann, 1948) with the skin and lip forming one and the tongue and tonsil the other end of the scale. For the same type of treatment and the same clinical stage of the disease the cure rate of differentiating squamous cell carcinomas is significantly greater than that of anaplastic tumors. This is not an unexpected finding since (a) tumors showing some differentiation before treatment are more likely to respond to irradiation with an increase in extent and degree of differentiation than anaplastic tumors; (b) for the same volume of tumor tissue the number of viable cells is smaller in differentiating cancers than in anaplastic tumors which contain no differentiating and non-viable cells which are usually of larger size than the small undifferentiated cells. For a given dose of radiation the chance of cells escaping lethal injuries increases with the number of cells. This has been shown in numerous radiobiological experiments from bacteria to ascites cell tumors. It is a well recognized experience of clinicians that small tumors at a given site are more amenable to radiation treatment (by the same dose) than large lesions, and cure rates for carcinoma of the cervix are much greater even locally in early than in late stages of the disease.

Bibliography

1. Cherry, C.P.: Brit. J. Radiol. 30:239, 1957.
2. Dominici, H. & Barcat, A.: Arch. Elect. Med. 15:835, 1907.
3. Friedman, N.B. & Brutz, E.: Cancer 2:1060, 1958.
4. Friedman, N.B., Sargent, J.A. & Brutz, E.: Canc. Res. 15:479, 1955.
5. Glücksmann, A.: Brit. J. Radiol. 21:559, 1948.
6. Hall, J.W. & Friedman, M.: Radiol. 50:318, 1948.
7. Hamperl, H. & Schwarz, G.: Strahlentherap. 24:607, 1927.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

PROGNOSIS BASED ON TUMOR TYPE AND PRESENCE OF LYMPHATIC EMBOLI

ALFRED GLÜCKSMANN AND CORA P. CHERRY

Cambridge, England, U.K.

The relation of the histological type of tumor to the year survival rate of patients with cancer of the uterine cervix is shown in Table I. In this series of cases only squamous cell carcinomas are considered, and the differentiating tumors (comparable to Broders Grades I and II) are characterized by the relative uniformity of cell strains, regarding size and chromatin content, the capacity of differentiation (i. e., keratinization) of a great proportion of cells even in young foci, the regular stratification of tumor strands and the associated presence of a well-developed cellular and fibrous stroma. Anaplastic tumors (comparable to Broders Grades III and IV) present a more varied appearance regarding cell strains, size of cells and nuclei and hyperchromatosis; they form irregular strands with little stratification and central necrosis, and only a small proportion of cells produce a low-grade keratin. In most of these tumors the stroma is poorly developed. Table I shows that even for the same clinical stage there is a significant difference in the radiocurability of differentiating and anaplastic squamous cell carcinomas. The only exceptions are Stage IV cases where the difference is not statistically significant.

TABLE I

FIVE-YEAR SURVIVAL RATE OF 898 PATIENTS WITH FULLY TREATED SQUAMOUS CELL CANCERS OF THE UTERINE CERVIX.			
Stage	Tumor type		Difference
	D (338)	A (560)	
1	96%	68%	28 ± 8.1
2	71%	50%	21 ± 5.4
3	44%	31%	13 ± 4.8
4	0%	9%	9 ± 4.7
All	55%	38%	17 ± 3.4

D = differentiated tumor type

A = anaplastic tumor type

Anaplastic tumors have a greater tendency to spread, and very frequently lymphatic emboli can be detected in a biopsy. Table II gives the incidence of lymphatic embolism as assessed on the pre-treatment biopsy and of lymph node metastasis as assessed in the specimen obtained by a Wertheim's operation on Stage 1 and 2 cancers of the uterine cervix. The anaplastic tumors have a significantly greater tendency to lymphatic embolism and lymph node metastasis than the differentiated tumors. Since lymph node involvement prejudices the result of local radium treatment and of surgery, the presence of lymphatic emboli has a prognostic significance.

TABLE II

LYMPHATIC EMBOLISM AND LYMPH NODE METASTASIS					
	No. of cases	% emboli	Difference	% metastasis	Difference
All cases	306	38 ± 2.8	-	42 ± 2.8	-
Emboli present	118	-	-	63	} 34 ± 5.6
Emboli absent	188	-	-	29	
Differentiating tumors	83	24	} 20 ± 5.7	20	} 30 ± 5.5
Anaplastic tumors	223	44		50	

Mixed carcinomas of the uterine cervix are found in about 10% of all cases. They contain squamous as well as mucin-secreting cells or cells which produce pseudo-mucin which can be detected with the periodic acid-Schiff technique. In the most mature form a differentiated squamous cell strain in separate strands is associated with a well differentiated columnar cell strain, while the most anaplastic forms are characterized by the appearance of signet ring cells or cells with a glassy cytoplasm and large nucleoli. Though the mature forms respond better to radiotherapy than the anaplastic types, the over-all cure rate is very low as seen in Table III. This Table records the results obtained in a separate series of 709 cases and shows a decreasing five-year cure rate in the order of differentiating squamous, anaplastic squamous cell cancer, adenocarcinoma and mixed cancers for the whole group as well as for Stages I and II separately.

TABLE III

HISTOLOGICAL TYPE OF CERVIX CARCINOMAS AND CURE BY RADIUM TREATMENT								
	D		A		C		M	
Type	No.	%	No.	%	No.	%	No.	%
Stages								
1 - 4	130	<u>49</u>	485	<u>17</u>	22	<u>9</u>	72	<u>3</u>
1	57	79	102	33	5	(40)	19	5
2	57	28	232	19	14	0	32	3
1 + 2	114	<u>53</u>	334	<u>23</u>	19	<u>10</u>	51	<u>4</u>

D = differentiated squamous cell carcinoma.

A = anaplastic squamous cell carcinoma.

C = adenocarcinoma

M = mixed carcinoma (adenoacanthoma).

No. = total number of cases.

% = percent cured, for five or more years.

It is thus possible to base a statistical but not an individual prognosis on the histological type of tumor as seen before beginning treatment and on the presence of lymphatic emboli. Since the histological type of cancer influences the treatment result and since the incidence of tumor types varies with the clinical material in time as well as with locality and social status of the patients, it is important to relate cure rates for cervical cancers to their histology.

Bibliography

1. Cherry, C. P., and Glücksmann, A.: *Cancer* 8:564, 1955.
2. Glücksmann, A.: *Brit. J. Radiol.* 29:483, 1956.
3. Glücksmann, A., and Cherry, C. P.: *Cancer* 9:971, 1956.
4. Glücksmann, A.: (1958) *Acta Union Internationale contre le Cancer* 14:358, 1958.

DISCUSSION

HANS F. BETTINGER, Melbourne, Australia:

In this contribution, Glücksmann and Cora Cherry endeavour to find a suitable basis for the comparison of cure results from various institutions. They suggest that "cure rates for cervical cancer should be related to their histology." Two objections seem obvious: the first is that histology is only one of the many variables that determine the outcome of a cancer case, and if the notoriously unreliable clinical staging is used as cross reference the objection becomes still stronger. The second objection must be raised against the division of squamous cell carcinomas of the cervix into differentiating and anaplastic ones. Quite apart from the semantics (the two words seem to refer to different frames of reference), if one were to make a graph with regard to the frequency with which the various forms of squamous cell carcinoma occur, one would not get a straight line which could be conveniently cut at some point to give two groups. The result would be the "normal" (bell shaped) curve with the tumors with an intermediate degree of differentiation forming a majority between the two extremes. If Broders's four groups have failed to gain general recognition it would be even more so with these two groups. How great the difficulties of such division are is best illustrated by the authors' tables. In the series of Table I the ratio of differentiating to anaplastic carcinomas is very roughly one to two. In Table III, equally roughly, one to four. What accounts for this difference?

There is one other question even if it is a pedantic one. It is realized that lymphatic embolism is a term often used for the phenomenon the authors describe, but is it really an embolism or is it not rather lymphatic permeation?

JORGE CAMPOS R. de C., Lima, Peru:

The authors find that the histologic type of cervical carcinoma is a good way of knowing in advance, statistically speaking, the response to radium therapy; although this criteria does not always have value in individual cases.

Corresponding by their results show, cervical carcinoma could be classified, histologically, into four types:

1. Differentiated squamous 2. Anaplastic squamous 3. Adenocarcinoma and 4. Muco-epidermoid. This classification seems to have more practical value than Broder's classification.

Our experience is based on a smaller series than Glücksmann and Cora Cherry's (89 cases, all of squamous and muco-epidermoid types), but there is a complete agreement with their results.

PIERRE DENOIX, Villejuif, Seine, France:

The evidence concerning the possible relationship between the presence of lymphatic vessel emboli at the site of the tumor and invasion of the lymph nodes as presented by Glücksmann and Cora Cherry, is extremely interesting. They apparently disagree, however, with my own findings in carcinoma of the breast (1) which disclose that there is no relationship whatsoever between lymphatic vessel emboli and the prognosis.

It is possible, however, that the fact that we deal here with carcinoma of the uterine cervix explains the difference in behavior. Table II of the authors considers only the relationship between the presence of emboli and the presence of metastases in lymph nodes. It would have been interesting to go further and to study the relationship between the presence of emboli and the long-range prognosis, and not to refrain ourselves, as the authors did, with the assumption that the presence of metastases in the lymph nodes represents poor prognosis. As a matter of fact, it is known that not all cases with lymphatic emboli have the same uniformly poor prognosis, and that rather there exists a scale of possibilities, depending upon the extension of this lymph node invasion. It would, therefore, be very interesting to know more about the relationship between the long-range prognosis and the following four categories:

1. Emboli and lymph node invasion
2. Emboli without lymph node invasion
3. No emboli but lymph node invasion
4. No emboli and no lymph node invasion

In the above paper (1) on breast carcinoma, these four categories were considered and the relationship is shown as follows:

Malignant Breast Tumors	Number of Cases	Apparently Cured After Three Years Observation (Percent)
1. Emboli and lymph node invasion	47	51
2. Emboli without lymph node invasion	39	70
3. No emboli but lymph node invasion	70	47
4. No emboli and no lymph node invasion	160	72

Bibliography

1. Denoix, P. F.: Monographie de l'Institut de l'Hygiene, No. 5., Paris, 1957.

CLAUD W. TAYLOR, Birmingham, England, U. K.:

The relationship between histological grade of squamous-cell carcinoma and prognosis is borne out by personal observations. In specimens removed at Wertheim's hysterectomy subsequent to radium therapy for carcinoma of the cervix (Dobbie, 1955) we found residual cervical carcinoma in 24 per cent of differentiated and in 57 per cent of anaplastic tumors; the pelvic lymph nodes were involved by carcinoma in 32 per cent and 56 per cent respectively. Lymph node involvement, however, depended mainly on the clinical stage of the disease, being 15 per cent in Stage I and 47 per cent in Stage II.

The detection of lymphatic emboli in biopsy of the primary growth needs elucidation. The stromal lymphatics are invaded by carcinoma as it grows; embolism implies detached particles of growth some distance from the primary source. Demonstration may require a large and deep portion of tissue.

Mixed carcinoma we regard as a variant of adenocarcinoma and should not be confused with mixed mesodermal tumor. The term "mixed tumor" implies that one component is heterotopic to the location in which the tumor is found (Taylor, 1958). Squamous epithelial cells are not rare in the endocervix and endometrium. Tumors arising from these glandular epithelia frequently contain squamous cell areas. In our experience adenocarcinomas in general respond more poorly to irradiation than squamous cell tumors.

The authors rightly emphasize that histological grade is not a reliable guide to irradiation response in an individual case. Carcinoma in situ and microcarcinoma may comprise undifferentiated cells and the prognosis be extremely good; on the other hand some examples of clinical Stage IV carcinomas are well differentiated but the outcome is hopeless.

Bibliography

1. Dobbie, B. M. W.: J. Obst. Gyn. Brit. Emp., 62:764, 1955.
2. Taylor, C. W.: J. Obst. Gyn. Brit. Emp., 65:177, 1958.

CLOSING REMARKS

ALFRED GLÜCKSMANN and CORA P. CHERRY:

Dr. Bettinger's question, "What accounts for the difference in the proportion of anaplastic and differentiating tumors in Tables I and III?" is indeed our central problem. The cases in these Tables were treated at different centers and are clearly distinguished by incidence of tumor types, distribution of stages and cure rates even for the same stage. Though clinical staging and histological grading are of limited accuracy, they are none the less closely related to cure rates and are thus of prognostic significance on a statistical as distinct from an individual basis. In a recent analysis of about 600 cases treated at a third center we found a close correlation between cure rate and clinical stage, between cure rate and Broder's grade for every clinical stage, but not between cure rate and radiation dose (range 5000 r to

9000 r at Point A). In brief: the higher the clinical stage and, within each clinical stage, the higher the Broder's grade, the lower was the cure rate. Our problem is to find various parameters to assess variations in the clinical material at different centers and to determine the factors which influence the cure of the patient. Histology of the tumor and clinical stage are two of these factors and it seems important to know whether and why cases presented at various centers in the same country or in different countries vary in their response to similar or modified treatments. Far from showing the difficulties of histological typing or grading, Tables I and III illustrate strikingly the important differences in the clinical material.

We agree with Taylor and Bettinger that tumor cells may be located in patent lymphatics by either permeation or by embolism. Serial sections show that the latter process is by no means rare. The main point of finding tumor cells in patent lymphatics is not how they arrive there but the likelihood that such cells are carried further by positive or more frequently by negative pressure in the lymphatic system. Table II and Denoix's Table show a good correlation between the presence of emboli and node involvement: in Denoix's series node involvement occurs in 55% of cases with emboli as against 30% without emboli. The incidence of embolism varies at different sites and is lower at the vulva for instance, though there the cases with emboli have an even greater likelihood of node involvement. Since embolism is a discontinuous process and not all emboli seed the lymph nodes, the correlation shown in Table II and by Denoix is quite striking evidence of the importance of embolism.

Denoix's failure to find (for breast cases) a prognostic significance in the presence of emboli though node involvement has a grave portent. For the cervix too (Clinical Stages I and II) node involvement decreases the chance of cure, but presence of emboli (without node involvement) has only a slight effect on cure rate if all cases are lumped together. On the other hand, in Stage I cases treated by radium plus surgery the five-year survival rate is only $33 \pm 6.8\%$ when emboli are present and $66 \pm 5.7\%$ when they are absent (difference $33 \pm 8.9\%$). For Stage II cases the greater likelihood of node involvement and the discontinuous nature of embolism obscure the prognostic significance of emboli as they do in the breast cases of Denoix. The argument that embolism is related to node involvement, that node involvement depresses the cure rate and the conclusion that embolism has a prognostic significance remains valid even if it can be demonstrated only for early cases.

We are glad to have Taylor and Campos confirm the prognostic significance of histological typing of tumors. The relation between histological type and clinical stage of a tumor will be discussed in the following topic of this Symposium.

We have to disagree with Taylor on the mixed carcinomas (not tumors!) of the cervix. These cancers may be derived simultaneously as separate strains from the squamous and the columnar epithelium and retain the characters of both types of carcinoma or originate from a stratified columnar surface epithelium at the os histologicum and be capable of either squamous or columnar or of a 'mixed' differentiation. There is evidence for both these possibilities. The mixed carcinomas are distinguished from the squamous cell and the columnar cell carcinoma of the cervix by their histology, their histochemical reactions, their relation to pregnancy and their response to treatment.

PROGNOSIS BASED ON SERIAL BIOPSIES

ALFRED GLÜCKSMANN AND CORA P. CHERRY

Cambridge, England, U.K.

Not all differentiating tumors respond to radiation treatment nor do all anaplastic cancers fail to do so, i. e., the structural anaplasia is not always a measure of the functional anaplasia of a tumor. To give a prognosis for the individual case it is necessary to test the response of the cancer. This can be done by taking serial biopsies from the growing edge of the tumor before treatment and at suitable intervals during treatment.

For cancers of the uterine cervix, two and at most three biopsies are required for this purpose. No untoward effects with regard to spreading of the disease and local response to radiation have been observed in well over 3000 cases. The taking of the biopsies, however, requires skill, care and experience, and in order to yield reliable information it is necessary to fix the samples properly and to avoid formalin fixation at all costs.

The interpretation of changes induced by radiation can be made only in relation to the dose given and the time interval. The persistence of unchanged foci characterizes a refractory tumor. Increases in cell size and the appearance of monster cells may often be an impressive sign of radiation injury but may constitute an only transient effect from which recovery is possible.

Three distinct types of response in individual tumors can be seen:

1. a favorable response in which the proportion of differentiating and degenerating cells increases at the expense of resting and dividing cells. These changes usually occur fairly quickly and may be accompanied by a fibrous or fibro-cellular reaction in the stroma of the tumor.
2. an unfavorable response in which there is little change in the proportion of resting and mitotic cells, some increase in degenerating cells but little or no alteration in the number of differentiating cells.
3. a partial and ultimately unfavorable response in which there is an initial slight increase in the proportion of non-viable to viable cells; but this is not maintained as treatment progresses.

For a favorable prognosis the proportion of viable cells should be reduced to less than half by a third of the total radiation dose and to less than a third by half the dose. The proportion of viable and non-viable tumor cells can be estimated visually without cumbersome cell counts.

Table I relates to the histological prognosis given within the first fourteen days of treatment for a series of some 1450 patients with the clinical result after five or more years and also shows that a correct prognosis was given in 86% of the cases.

In the majority of cases a definite prognosis can be given, but sometimes the biopsy material is insufficient either with regard to timing, number or the site of the sample, and occasionally the changes induced by radiation are equivocal. The number of such instances varies inversely with the enthusiasm and ability of the clinician who takes the biopsies.

TABLE I

HISTOLOGICAL PROGNOSIS AND FIVE-YEAR SURVIVAL RATE					
Stage	Favorable prognosis		Unfavorable prognosis		Prognosis correct
	No.	% 5 year survival	No.	% 5 year survival	%
I	136	83	164	19	82
II	167	68	575	12	83
III	33	72	300	10	88
IV	2	100	76	8	94
	338	74	1115	13	86

Table II records a "doubtful" prognosis in 16% of 366 cases who were operated on following radiotherapy, largely because of an unfavorable histological prognosis. As "special controls" cases with favorable prognoses are included and as "general controls" cases with doubtful prognoses and only with a pretreatment biopsy. All patients were in clinical Stages I and II. In 83% of the cases with a favorable prognosis no histological evidence of carcinoma was found at the cervix though up to 72 blocks per case were sectioned; of the cases with an unfavorable prognosis 89% had viable tumor tissue at the cervix and an additional 6% had involved lymph nodes. In the cases with a doubtful prognosis and those not subjected to serial biopsies, 55 and 60% respectively had cancer at the cervix and an additional 12 and 10% had involved lymph nodes.

TABLE II

HISTOLOGICAL PROGNOSIS AND HISTOLOGICAL FINDINGS AT OPERATION FOLLOWING RADIATION TREATMENT											
Biopsies		Serial						Single		Totals	
Prognosis		Favorable		Unfavorable		Doubtful					
		No.	%	No.	%	No.	%	No.	%	No.	%
Cervix	Nodes										
+	+	8	12	90	44	14	25	10	25	122	34
+	0	3	5	93	45	17	30	14	35	127	35
0	+	14	22	12	6	7	12	4	10	37	10
0	0	39	61	10	5	19	33	12	30	80	22
Totals		64		205		57		40		366	

+ = cancer present

0 = cancer absent

Measured by the histological findings at operation the favorable prognosis was correct in 83% and the unfavorable correct in 90% of the patients. Persistent pelvic cancer occurred most frequently in patients with an unfavorable prognosis and less frequently in those with a favorable prognosis, while the findings in the two groups of general controls were intermediate.

When surgery is the only useful alternative to radiation treatment, the serial biopsy method should be applied only to clinically operable cases. It is very useful as a research method for the study of radiation effects and alternative therapeutic investigations.

Bibliography

1. Glücksmann, A.: Recent Advances in Clinical Pathology. London, 1947, J. & A. Churchill Ltd.
2. Glücksmann, A.: Acta, Union Internationale Contre le Cancer 14:358, 1958.

DISCUSSION

HANS F. BETTINGER, Melbourne, Australia:

The serial biopsy method seems to give excellent results in the hands of Glücksmann and Cora Cherry. So does, by way of comparison, the determination of SR and RR in the hands of Ruth Graham. However, there are far too few reports on investigations which have attempted to assess, in sufficiently large series, the accuracy of either method in the hands of other workers. There is to my knowledge no report in the literature at all on a series in which both methods have been tested in the same group of patients. An institution that would carry out such an investigation on a large scale would earn the gratitude of all the workers in this field.

JORGE CAMPOS R. de C., Lima, Peru:

The therapy of cervical cancer by radium as well as by surgery seems to have reached its greatest possibilities since the statistics of the best medical centers do not demonstrate significant improvements in the last few years. That is why it is interesting to investigate new methods which would improve the present results. The combination of radiotherapy and surgery would seem to be a good answer if it could be demonstrated scientifically which case would have a better response to radium therapy and which would be resistant to radium before a relapse would occur showing too late the resistance to radiation therapy.

The serial biopsy method of Glücksmann and Cora Cherry can be done in any specialized center, and since the authors' experience shows that one can take two or three biopsies without harming the prognosis, it should be attempted in order to improve the present results of therapy for cervical carcinoma.

PIERRE DENOLX, Villejuif, Seine, France:

The paper by Glücksmann and Cora Cherry on prognosis of carcinoma of the uterine cervix based upon serial sections of the uterus is extremely interesting. The figures given therein prove the value and prognostic certainty of this method.

If one tries to compare this method with the cytochemical one of Herovici, it immediately becomes evident that the latter is equally good and, although more optimistic as to the results (1), there is nothing that indicates the superiority of one over the other.

It seems that the essential problem concerning these two tests would be to learn whether or not they are in some way statistically correlated.

Each test represents a considerable prognostic criterion. In the event that they have no statistically appreciable connections, one should, by taking each into account, achieve a remarkable prognostic certainty.

Therefore, it would be desirable to proceed with these investigations by using both methods. It is my hope to see these correlative studies carried out in the future.

Bibliography

1. Herovici, C. and Kritter: *Bulletin du Cancer* 42:29, 1955.

S. B. GUSBERG and GRACE HERMAN, New York, New York, U.S.A.:

The three distinct types of response defined here correspond closely to the RST designations we utilize and to those of other workers in this field: the favorable response, the unfavorable response and the partial or mixed response. It is interesting that cytologically in an RST good response, 65% or more of the tumor cells show significant radiation effect by differential count.

One must place great emphasis on the persistence of unchanged or minimally altered foci and read as RST moderate the response that shows variation, i. e., some areas with rather marked radiation effect, but a significant amount of tumor with only minimal alteration. In our present program those Stage I and II lesions read as RST poor or RST moderate are transferred without delay from a radiotherapeutic to a surgical plan of therapy following RST evaluation.

Our biopsy technique utilizes samples from fresh tumor areas near but not at the growing edge of the tumor taken with a punch or the sharp Gusberg curette. In the majority of Stage I lesions, and in some of the Stage II lesions, only one post-radiation biopsy is required.

This method has given us a prognostic accuracy of approximately 75% with respect to the immediate healing of the tumor. This enables us to support the view of Glücksmann and Cora Cherry that serial biopsy can furnish a significant prognostic tool.

CONSTANTIN HEROVICI, Villejuif, Seine, France:

For the past two years we have adopted in our laboratory the test of Glücksmann. Together with histochemical studies of the tumor and its underlying tissue the test allows a histoprognosis of carcinoma of the uterine cervix. Thus far we agree with the report of Glücksmann and Cora Cherry. We also consider the absence of the tumor in posttherapeutic specimens a great disadvantage, an incidence which happens in one out of three cases. In repeated negative biopsies, one still encounters this inconvenience one out of two times. But there are still many specimens left in which the histoprognostic study can surely be done from the study of the reaction in the surrounding benign tissues.

In all favorable cases where we find at the site of the tumor the criteria of Glücksmann, we find a decrease of nuclear DNA, an increase of cytoplasmic RNA, as well as deposits of glycogen at the site of viable tumor tissue, which histologically does not show any radiation response.

As far as the modifications of the connective tissue in the sense of maturation of the collagen are concerned, we feel that this is a delayed phenomenon which begins having prognostic value only two months after irradiation.

CLAUD W. TAYLOR, Birmingham, England, U.K.:

Response of a tumor to irradiation must be assessed individually; for this purpose it is essential that the initial biopsy should contain young tumor foci and that subsequent biopsies show comparable areas. Many a pathologist finds that the specimens he receives do not conform to these criteria; my own experience has been that in at least half the cases the material has been unsatisfactory for assessment. Not all clinicians are willing or able to supply the necessary large and deep biopsies required; interpretation of postirradiation biopsies is often obscured by necrosis.

With good specimens and good fixation the degree of tumor response can usually be assessed. Examination of a subsequently excised uterus usually shows correlation with the assessment. Ultimate prognosis, however, so often depends on whether or not the pelvic lymph nodes have been invaded by the carcinoma; the primary growth may show a "favorable" response and indeed be "cured" but the patient may still die of metastases. In this respect the figures given by the authors in Table I might be elucidated. In cases of clinical Stage I they find slightly more tumors to be unfavorable than favorable (164 : 136) whereas in Stage II the ratio is much larger (575 : 167), in Stage III it is even larger (300 : 33) and in Stage IV larger still (76 : 2). This progression of unfavorable response linked so markedly with clinical stage might lead to the suspicion that histological assessment has been influenced by the stage of the disease. I am sure the authors will answer this point satisfactorily, but it needs answering.

CLOSING REMARKS

ALFRED GLÜCKSMANN and CORA P. CHERRY:

Most of the points raised by Taylor, Denoix, Herovici, Gusberg and Grace Herman have been dealt with in the Symposium, in the topic entitled "Histological Criteria of Radiation Response," of this issue. Their remarks, like those of Campos, testify to the value of histological or histochemical assessment. Taylor, Herovici, Gusberg and Grace Herman stress the difficulties of getting adequate biopsies. This is an important matter requiring skill and advanced planning and should not be left to the mercy of beginners. Since the appearance of the lesion is altered by radiation treatment, the choice of a suitable region for biopsy at later stages of the treatment period is difficult. It is useful to make a sketch of the extent of the tumor at the beginning of treatment and to decide about the location of subsequent biopsies at that time.

Taylor points out that the proportion of unfavorable cases increases with the stage of the disease. This is to be expected, as in advanced tumors even the carcinoma of the primary site fails to respond. In other words, tumors which tend to spread are also very frequently refractory to treatment. This can be shown particularly clearly for oral cavity cancers with involvement of regional lymph nodes. Postmortem examinations show that cancer persists at the primary site in about 75% of the cases. We have reported similar figures for cancer of the cervix, though in this localization the adequate radiation treatment of the nodes presents formidable problems. The quantitative aspects of number of tumor cells influencing cure rates and with it the incidence of unfavorable cases have been mentioned in this issue as stated previously.

We welcome Bettinger's suggestion of a large scale comparison of cytological and histological assessment of radiation response. An analysis by Ruth Graham and by us of a few cases of Mr. Stanley Way produced surprisingly good agreement although one method uses normal cells and the other tumor cells.

DO SERIAL BIOPSIES DISTURB THE HEALING PROCESS OF THE IRRADIATED CERVICAL CARCINOMA?

HANS-LUDVIG KOTTMEIER

Stockholm, Sweden

The healing process of an irradiated tumor is dependent upon the destruction of the malignant cells by beta and gamma rays and upon the condition and reaction of the normal tissue. For many years it has been the experience of the Radiumhemmet that any interference with the healing process of the tumor by rupturing the growth will unfavorably influence the prognosis. In 1916 and 1925, Heyman took serial biopsies from cervical carcinoma during the course of irradiation. He attributed the poor results in these two series, 7.5 and 13.6% five year cure rate in 53 and 140 cases respectively, to the biopsies taken. Berven had similar experience with irradiation of tonsillar and buccal carcinoma.

For many years Heyman laid stress upon the significance of intra-uterine radium in the treatment of uterine carcinoma. Sometimes this application was difficult, and searching for the cervical canal caused ruptures in the growth. Experience has shown that the application of radium in such a manner has caused local infection and that the ultimate prognosis in these cases has been extremely poor.

The significant and excellent studies by Glücksmann have been an approach to the recognition of radioresistance and radiosensitivity in carcinoma of the cervix. At the Radiumhemmet we do not have sufficient experience for forming a personal opinion on the value of serial biopsies in judging the radio-curability. In recent years we have, however, in some cases of cervical carcinoma, taken biopsies from the edge of the growth during the course of irradiation. The biopsy has been taken with a scalpel, scissors or similar instrument. In about 40% of these cases we have observed unpleasant reactions - infections and necroses - in the area of the biopsies, complications which as a rule we do not see and which, perhaps incorrectly, we have attributed to interference with the healing process due to taking the large biopsy. The following case history may elucidate my viewpoint.

Case NO. 431/1944. Thirty-three years of age. Gravid II. Postcoital bleeding for three months. An epidermoid carcinoma (Grade II, of the anterior lip, the size of a plum) was diagnosed. The case was classified as carcinoma of the cervix, Stage I. Further studies did not reveal any abnormalities. Radium was applied according to the current Stockholm technique: April 23, 1955 - 53 mg. of radium into the uterine cavity for 25 hours; May 15, 1955 - 200 mg. of radium into the uterine cavity for 15 hours; April 23, 1955 - an additional application of 70 mg. of radium into the vagina for 25 hours. In June, 1955, x-ray therapy was administered towards the lateral pelvic wall from two anterior and two posterior portals. A dose of five times 400 r was given to each field.

Following the first application of radium, biopsies were taken once a week with a punch from the right edge of the growth and the right fornix during a course of four weeks. In May, 1955, the patient began to suffer from severe pain in the right pelvis and the right leg. Examination revealed a deep necrotic ulcer in the right fornix penetrating to the parametrium. For a long time I considered the necrotic mass to be cancer, although the smear and the biopsies taken during the summer and fall, 1955, did not show any malignant cells. The necrotic ulcer increased considerably in size during the first four months. In December, 1955, however, the symptoms disappeared and the necrosis slowly healed. Since 1956 the general condition of the patient has been improved. Today, however, the patient possibly has a metastasis on the right pelvic wall. The fact that a hydronephrosis and a hydroureter have developed on the right side supports this presumption.

DISCUSSION

ALFRED GLÜCKSMANN, Cambridge, England, U. K. :

In well over 3000 cases of cervical cancer with serial biopsies collected from eight large clinical centers we rarely encountered infection and necrosis attributable to repeated incisions and found no evidence for the spreading of the tumor by these procedures. We do not deny that both risks exist, but they seem to be at the most less serious than other accepted practices.

There is no convincing proof that radiation within the therapeutic dose range retards wound healing nor that surgery prejudices the healing of radiation reactions, (for example, the widely practiced postoperative radiotherapy for breast cancer). Single incisions of infected cancers for routine diagnostic purposes, large ring biopsies and conizations should likewise spread infection but are generally considered harmless. The presence of virulent infective organisms may cause complications and it is feasible that the coincidence of infection with a particularly virulent organism and the taking of serial biopsies is responsible for the 40% infection plus necrosis rate in Kottmeier's series.

The risk of spreading the tumor by repeated biopsies has been analysed in a series of 213 cases and no correlation was found between the incidence of metastases and the number of biopsies (1). Lymphatic emboli are not infrequently encountered in routine single biopsies of cervical cancers but such emboli do not always, or even often lead to the establishment of metastases (2).

Young and co-workers (3) contend that embolism is due to an increase in tissue pressure over intravascular pressure. Palpation of tumors and the dilatation of the cervical canal prior to the insertion of radium applicators increase the tissue pressure and thus the risk of embolism. A similar increase in pressure is caused by incision, but the outward flow of blood and lymph is likely to drain away any embolic cells.

In animal experiments the taking of biopsies failed to increase the metastatic spread of tumors (4), while the manipulation of experimental tumors did so (Tyzzer quoted by Ewing in 'Neoplastic Diseases'). There is also experimental evidence that the irradiation of spontaneous and of transplanted animal tumors may increase the rate of metastases (4, 5, 6).

Both practical experience on thousands of cases and experimental evidence thus suggest that the risk of spreading a tumor by serial biopsies is negligible and probably less than that due to palpation and manipulation of the tumor.

The role of dose, dose-rate, field size and other physical factors of irradiation as well as of individual sensitivity on the induction of radiation necrosis are too well known from clinical experience and animal experiments to need any further comment.

Bibliography

1. Cherry, C. P. and co-workers: J. Obst. Gyn. Brit. Emp. 60:368, 1953.
2. Cherry, C. P. and co-workers: Cancer 8:564, 1955.
3. Young and co-workers: J. Path. Bact. 62:293, 1950, & 62:313, 1950.
4. Kaas, S.: Cancer Research 13:744, 1953.
5. Van Essen and co-workers: J. Nat. Canc. Inst. 12:883, 1952.
6. Kaplan and co-workers: J. Nat. Canc. Inst. 9:407, 1948.

S. B. GUSBERG and GRACE HERMAN, New York, New York, U. S. A. :

We have taken serial biopsies for radiosensitivity evaluation in 162 cases of cervical carcinoma. These biopsies were taken from fresh tumor areas near but not at the growing edge of the tumor with the conventional Gaylord punch or with the sharp Gusberg cupped curette. No significant infection or necrosis has resulted. Stage for stage the salvage rate of these biopsied patients is comparable to a control group of patients similarly treated but not subjected to serial biopsies. We believe that serial biopsies obtained in the manner described above has had no detrimental effect on healing as ascertained from a comparison of the cure rates in our institution for each stage of the disease. Large biopsies taken through the growing edge of a tumor into normal tissue could be responsible for spreading the tumor or inducing necrosis, or even interfering with the containment of the tumor by the stromal reaction. Our smaller biopsies taken within the tumor have been adequate for tissue and tissue-smear evaluation. There is no doubt that surgical gentleness in handling tumors, whether the therapeutic modality is radiation or radical surgery, will diminish spread and enhance the efficiency of treatment.

CLOSING REMARKS

HANS-LUDVIG KOTTMEIER:

For many years it has been well known by all radiotherapists that a serious infection will unfavorably influence the radiocurability and may increase the risk of local necrosis. The studies by Philipp

and Ruge are well known in this respect. At the Radiumhemmet we have not been able to confirm the studies by these German authors, that the hemolytic streptococcus especially, were causing serious complications. On the other hand several years ago I was able to demonstrate the relationship between radio-curability and local infection. The observation was made that the necroses in our cases with large serial biopsies appeared in the area of these biopsies.

At the Radiumhemmet we disagree with Glücksmann's remark that "there is no convincing proof that radiation within the therapeutic dose range retards wound healing. ----conizations should likewise spread infection but are generally considered harmless." In recent years we have, at the Radiumhemmet, performed conizations or very large biopsies, in cases in which a carcinoma in situ was diagnosed in curettings or small biopsies, provided that colposcopy or further examination did not reveal any clinically visible cancer. In about 17% of such removed cones an obvious invasive cancer has been diagnosed by semiserial sections. In about 20% of the cones, a questionable invasive carcinoma was diagnosed. Careful measurements were carried out. Although the dose applied has been decreased, with about 10% of the routine dose applied in Stage I cases in several of these early cases treated by radiation we observed an unpleasant necrotic crater in the tip of the vagina; a complication that we do not see in cases in which no major surgery is performed. We, therefore, have decreased the dose to 75% of the routine dose. Similar observations are reported in cases of local recurrences after major surgery in which radiation was given. At the Radiumhemmet, this has been our experience in cases of carcinoma of the endometrium, of the vulva and of the breast.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

THE METHOD OF PROGNOSIS AFTER IRRADIATION BY MEANS OF EXFOLIATIVE CYTOLOGY

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

Our first interest in radiation cell changes stemmed not from an attempt to establish a prognostic method but from an interest in increasing our diagnostic accuracy. In 1942 when I began to do exfoliative cytology, the majority of smears received were from the Massachusetts General Hospital tumor clinic. The majority of these patients had received radiation in the past for cancer of the cervix. In many ways it was a difficult group from a diagnostic point of view. The attempts to distinguish between radiated benign cells and true malignant cells did not always meet with success. There were seven patients whose smears had been called positive and in whom no recurrent disease was found after careful investigation. Vaginal smears were either called positive or negative (1). Meigs insisted that if Papanicolaou's method was correct the cytologist should be able to distinguish between benign and malignant cells. It soon became fairly obvious that we were misinterpreting radiated benign cells and considering them malignant. I need not emphasize that this remains a problem in some instances even today.

It occurred to us that one solution to this diagnostic problem was to take daily smears on patients with cancer of the cervix during their course of radiation therapy. It was our thought that if we observed the progressive changes as a result of radiation, we would then be able to recognize such changes months or years later.

Smears were collected on thirty-five patients during their course of therapy. When they were examined, it was apparent that we were dealing with two groups of patients. In one group there were progressively marked cellular changes and in the other the changes were few. This seemed to be a definite difference in biologic behavior. Almost immediately the question arose whether or not the two groups had received comparable doses of radiation. Perhaps the group in which few cellular alterations were present were obese patients. Since the primary treatment was x-radiation, it was possible that in such patients less radiation would reach the area of the cervix. The records of these cases were carefully reviewed by members of the radiology department. They concluded that the groups did not differ in the amount of radiation received at the cervix. Further investigation revealed that the patients whose smears contained cells with marked changes did rather well, while the majority of those with few changes had recurrence of their disease (2).

In this first investigation the radiation effect on benign cells was assessed in a qualitative fashion. Four gradations of response were considered from one plus to four plus. If any smear during the course of treatment showed a four plus response, the patient was considered to have a good response. This method with three-year results from the prognostic standpoint appeared in 1947 (2).

Considerable interest was shown in this prognostic method, but the results were criticized because of the qualitative estimation of radiation response. Originally, it was felt that it would be impossible to do accurate counts on vaginal smears, since the distribution of cells is rather poor. However, this assumption did not prove to be correct. To test the validity of differential counts on vaginal smears, fifteen smears from one vaginal secretion were prepared at the same time. Differential counts were done. The counts agreed well. Other studies such as this confirmed that differential counts would be in agreement if groups of over six cells were ignored (3). No agreement was possible if sheets of cells were counted.

The method used by our laboratory at the present time is as follows: Vaginal smears are obtained on every cancer of the cervix treated by radiotherapy three times a week during the entire course of therapy. At least 100 cells in every smear are counted differentially for the percentage of benign cells showing radiation change and for the total percentage of malignant cells, including those showing radiation effect and those with none. The benign cells showing radiation response are divided into the three cellular groups: superficial, intermediate and basal, and then subdivided into the four characteristic radiation

changes: vacuolization, nuclear change, size increase and multiple nuclei. These divisions are not really necessary from a prognostic standpoint, since it is the total percentage of change which determines whether or not a response falls into the poor or good category. We subdivide cells in this way because we are interested in finding out which change is the most critical.

By this method we have a curve of radiation response for each patient. Interpretation of the curve according to time and dosage will be considered in another section of this symposium.

Bibliography

1. Meigs, Joe V. and Graham, R.M.: Surg. Gyn. & Obst. 77:449, 1943.
2. Graham, R.M.: Surg. Gyn. & Obst. 93:767, 1951.
3. Graham, J.B., Graham, R.M. and Liu, W.: Surg. Gyn. & Obst. 99:555, 1954.

OLLE KJELLGREN Gothenburg, Sweden

At the Radiation Centre, Gothenburg, we have been analyzing radiation cell changes in the vaginal smear and their prognostic significance in irradiated cervical carcinoma since 1950, the total number of patients being about one thousand to date.

The radiation cell changes are judged according to criteria specified under the first topic of this Symposium entitled "Definition of Radiation Response on Normal Squamous Cells (RR Cells)."

A sample of fluid is aspirated from the upper part of the vagina with a Papanicolaou pipette, ejected on a slide, smeared with the pipette tip, fixed in equal volumes of ether and ethanol, and stained in accordance with Papanicolaou's original method.

The slide is examined under a light microscope of about 600 power. In a region sufficiently thin to permit single cells to be distinguished, every cell is inspected separately and classified with respect to radiation changes as specified in the first topic of this Symposium, clusters of five or more cells being ignored.

Some sources of error have been evaluated. For one of those, non-specificity of the radiation reaction, see the first topic of this Symposium. Another, "aspiration level," was studied by taking samples of vaginal fluid from just within the introitus as well as from the posterior fornix of 16 patients. In five of the 16 pairs of smears examined, the frequency of radiation changes in the smear from just inside the introitus was significantly lower than in the corresponding smear from the posterior fornix.

The error of estimation affecting the evaluation of the radiation changes in the smears was analyzed by re-examining 29 smears taken at random from the files. According to the formula enunciated by Dahlberg (1940), the standard error for a single determination is:

where N is the number of differences and d the difference between the two duplicate determinations. Application of this formula disclosed that the standard error was 3.3%.

$$m = \sqrt{\frac{\sum d^2}{2N}}$$

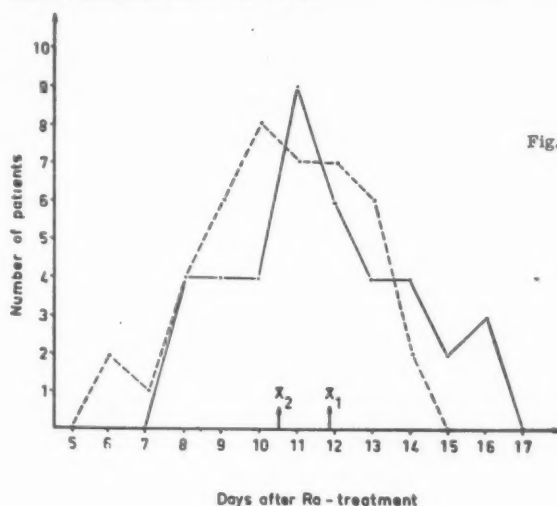


Fig. 1. Day for maximal radiation changes after Ra I (—) and Ra II (---). \bar{X}_1 = mean after Ra I; \bar{X}_2 = mean after Ra II.

With the modified Stockholm method we use, the maximal radiation reaction occurs 11.88 ± 0.47 (dispersion: 2.99 ± 0.33) days after the first radium application and 10.50 ± 0.30 (dispersion: 1.94 ± 0.20) after the second. Hence, a vaginal smear obtained 10 to 14 days after a radium application should, and in practice we feel it does, provide a reasonably accurate idea of the maximal level of the radiation reaction (Fig. 1).

A radiation reaction of 60% or better, that is, with at least 60% of the benign exfoliated squamous epithelial cells showing changes meeting the criteria specified in the first topic of this Symposium, is designated a good response; others are called poor responses. The prognosis is considered good for patients with a good response at any time after the first or second radium application and poor for patients with no good response to irradiation.

Analysis of the first 287 patients show that $51.6 \pm 2.9\%$ exhibited a poor and $48.4 \pm 2.9\%$ a good response to irradiation.

Bibliography

1. Kjellgren, O.: Acta Radiol. Suppl. 168, 1958.

LUIS MONTALVO RUIZ AND VICENTE JIMENEZ TEBAR

Madrid, Spain

Since 1947, when Ruth Graham (4, 5) published her first studies on the modifications of exfoliated cells of the vaginal epithelium after radiation in cases of uterine cancer, a series of publications (2, 3, 4, 5, 6, 7, 9) followed trying to establish a prognostic index based on these changes.

Most of the authors of recent publications agree with Ruth Graham. However, Limberg, Napp and Wilbrand (8) do not give prognostic value to the changes of the benign vaginal cells, but consider as more valid the post-radiation changes in the tumor cells. Pundel (10) seems to agree with these authors.

Julieta C. de Laguna's work (1) is also interesting, since she has studied the persistence of the Radiation Response (RR) as a prognostic index for carcinoma and observed a relationship between the persistence in the changes of the irradiated cells and the evolution of carcinoma of the uterine neck.

MATERIAL AND METHOD

We have studied cytologically 127 cases which had been radiated, almost all with radium. With the exception of 19 who did not return, they all (108) were examined every three months the first year and every six months in the following years for five consecutive years.

The method used was as follows: first smear, before radiation; second smear, seven days after receiving 2300 mgh.

Interpretation: Following Ruth Graham's view we undertook a recount of the exfoliated cells of the normal vaginal epithelium and noted the cellular anomalies and the percentage of cells in the smear.

We accepted as a good response a rate of over 70% and as a poor response a rate of under 60% of these cells. We gave a higher value to the reading of the second smear after radiation, that is to say, in the test ten days after receiving 4600 mgh, as in the first smear after radiation, we still perceived tumor cells and much cellular debris and leukocytes, which prevented us from obtaining the correct reading. What we especially observed is enlargement of the cell, vacuolization and multinucleation of the parabasal and intermediate cells. Later, we saw the superficial cells become bi-nucleated and enlarged. We prematurely observed the brown coloration in the parabasal cells.

RESULTS

Total cases studied and controlled . . . 108
With good RR 67
With poor RR 41

ALIVE	
With good RR	With poor RR
After 5 years 17	After 5 years 6
After 4 years 6	After 4 years 1
After 3 years 7	After 3 years 1
After 2 years 10	After 2 years 3
After 1 year 11	After 1 year 4
Total 51	Total 15

DEAD	
With good RR 16	With poor RR 26

Comment: Considering our results, we believe that studying the vaginal cytology after radiation of the neck cancer and following Ruth Graham's view, a prognosis can be established quite closely related to the clinical course.

Bibliography

1. Calderon de Laguna, J.: Proceedings, First Pan American Cancer Cytology Congress, 1957, Miami, Florida.
2. Cherry, C.P. and Glücksmann, A.: Cancer 7:504, 1954.
3. Ghilain, A. and Bouwer, W.F.: Gyn. and Obst. 51:309, 1952.
4. Graham, R.M.: Surg., Gyn. and Obst. 84:135, 1947.
5. Graham, R.M.: Gyn. and Obst. 84:165, 1947.
6. Graham, R.M. and Goldie, K.R.: Cancer 8:71, 1955.
7. Graham, R.M. and Graham, J.B.: Progresos de la Ginecologia Madrid, 1958, Editorial Cientifico Medica.
8. Limburg, H., Napp, J.H. and Wilbrand, U.: Geburtsh. u. Frauenlkh. 12:723, 1952.
9. Nielsen, A.M.: Acta Radol. 37:479, 1952.
10. Pundel, J.P., Bourg, R. and Gompel, G.: Diagnostic cytologique du Cancer Genital. Paris, 1954, Masson.

DISCUSSION

D. A. BOYES, Vancouver, British Columbia, Canada:

The prognostic significance of radiation response as described by Ruth Graham is again confirmed by two more groups of workers. Of the patients studied in our laboratory only 72 have been followed for five years. Of these, 64% show a good RR, the good having a 72% five-year survival and the poor a 20% five-year survival. We therefore feel that, although the technique is not easy, the observation, that certain cellular changes induced by radiation reflect prognosis, is probably correct. The value of this observation, however, remains to be proved. It has not yet been shown that the survival rates for those patients with a poor radiation response can be improved, and until this is shown, the worthiness of studying radiation response and similar prognostic techniques will not be known.

EMMERICH von HAAM, Columbus, Ohio, U.S.A.:

Ruth Graham's development of her prognostic method based on differential counts of exfoliated cells proves what keen observation of small details can achieve. The value of her method is again confirmed by Montalvo Ruiz and Jimenez Tebar in this Symposium, and Ruth Graham's method has become standard routine in many hospitals for the follow-up of radiation treatment of cancer of the cervix. Kjellgren's evaluation of sources of errors in prognosis by means of exfoliative cytology has shown a standard error of only 3.3% by the modified Stockholm method.

OLAF T. MESSELT, Oslo, Norway:

I have given elsewhere in this Symposium the results of my investigation of the method of Ruth Graham as to prognosis by means of RR, and can only confirm my opinion that the method is a valuable means in determining prognosis in the great majority of cases of cancer of the cervix treated by radiation.

HORST SMOLKA, Kiel, Germany:

Our material consists of cytological specimens which have been examined before, during and after irradiation treatment and the fate of which, now, after eight years, can be evaluated. We have the impression that cytology gives certain hints towards a poor prognosis only in cases with a faint radiation response of the benign epithelium. A marked radiation response of the cells does not necessarily speak for a favorable course of the disease.

The reasons for this discrepancy between the radiation response and the clinical course are of different kinds. In the first place, for a successful radiation treatment the size and the topographical extent of the tumor is important. Among our cases are a number of patients who have died in spite of good radiation response because the growth had already progressed too far. Therefore, in my opinion the grade of extension of the tumor has to be considered first in prognostic evaluations. The cytological evaluation can be applied only to tumors with the same degree of invasiveness.

Other reasons for the discrepancy between radiation response and clinical course are certain peculiarities of the tumor tissue, e.g., various degrees of differentiation, the resistancy of the connective

tissue and the amount of radiation which is effective on the normal tissue surrounding the tumor. In this regard the distance of the radiation carrier from the vaginal wall is of importance and this depends upon the width of the vagina and the stuffing of the vagina with tampons or plastic.

CLOSING REMARKS

RUTH GRAHAM:

To Dr. Boyes: I am of course pleased that Boyes has found such differences in survival rate between the good and poor responses. I would agree with him that because a method is shown to have prognostic value an increase in survival rate is by no means guaranteed. However, by this method one is able to recognize a group of patients where other measures should be attempted. Only time will tell whether surgery or modifying the response will result in a higher survival rate. Some progress is represented in the fact that we at least can decide toward what patients our efforts in this direction should be directed.

To Dr. Smolka: This discussion is rather vague. The comments are broad generalities on a number of subjects most of which are not under consideration in this section of the Symposium. Since no data, either number of cases, number which are considered good or poor responses or results are given, it is difficult to evaluate this statement.

OLLE KJELLGREN:

Like Smolka I have found that the prognosis is not absolutely good in cases with good radiation response and not absolutely poor in poor response cases. Details concerning this problem are found in the topic entitled "Results of RR-studies" in this Symposium. The importance of factors such as the radium dose and the distance of the radium applicator from the mucus membranes are discussed later in this Symposium.

LUIS MONTALVO RUIZ:

To Dr. Smolka: We think that his discussion is somewhat outside of the topic. We have limited ourselves to speaking only of the prognosis by exfoliative cytology. There is no doubt that it is possible to establish a prognosis of carcinoma principally based on histologic findings and general clinical evidence. One of us (Jimenez) presented a paper together with Nogales at the second meeting of Spanish gynecologists in December, 1957. In that paper, we studied in minute detail the roll of connective tissue and the histologic classification concerning the prognosis of uterine carcinoma. We will not discuss the matter at this time. However, further information may be obtained from the proceedings of the above-mentioned meeting published by Edicion de Toko - Ginecologia Practica, Londres 45, Madrid, 1958. We agree with Smolka that the grade and topographic extension of the tumor are important factors to be considered for successful therapy. We also have sixteen deceased cases having a good RR (radiation response) as can be seen in our table. Because of the aforesaid, it was pointed out at the conclusion of the main paper that by means of a cytologic method, a prognosis can be established following closely, but not exactly, the clinical course. We also agree with Smolka in that the cytologic prognosis has more value if it is applied to tumors of the same grade.

COMPARATIVE STUDIES OF EXFOLIATIVE CYTOLOGY AND HISTOLOGY AFTER IRRADIATION

JORGE CAMPOS R. de C.

Lima, Peru

We have studied 30 cases of carcinoma of the uterine cervix, six of Grade I, 12 of Grade II and 12 of Grade III. Their clinical evolution has been followed for three years. One case of Grade I, two of Grade II and seven of Grade III are dead or evidence active disease at this moment.

In every case cervical smears and cervical biopsies were taken before the patient received a four to five weeks course of x-ray therapy with an average dose of 3151 r. A second sample was taken for cytologic and histologic examination at the end of this treatment then radium therapy was given with an average total dose of 6056 r. Two weeks after that we took the third sample and six weeks later the fourth sample, both for cytology and histology of the cervix.

The purpose of this work is to study the cellular changes provoked by irradiation and its relationship to the clinical evolution of each case. At this time we are going to report only the relationship we found between the histological and cytological findings, according to the program of this Symposium, without discussing their prognostic value.

Observations: The cytological changes we find in the smears were observed both in the normal epithelial cells as well as in the neoplastic cells; they correspond to the changes described by Ruth Graham (1).

In the biopsies the response to irradiation is observed in the normal epithelium, in the neoplastic tissue and in the connective and vascular tissues. The more affected normal epithelial cells are those of the basal and intermediate layers. These changes consist of abnormal shapes, giantism, cytoplasmatic vacuolization, leukocytic infiltration and eosinophilia. The nucleolar response consists of increase of size and chromatin with bizarre atypical forms, lysis and leakage of the nuclei.

The neoplastic tissue with good irradiation response shows the same morphological changes as the normal epithelium. In other cases we could observe a deviation of the initial morphology toward a more mature differentiated form, with the predominance of the cytoplasm without giant cells and bizarre nuclei; we consider these cases as poor response.

After radium application it is very difficult to find tumor tissue for examination. When it is still viable and present in the cervix, it indicates a bad prognosis.

The changes of the cellular morphology found in the smears are comparable with those found in the cervical tissues. In 23 out of 30 cases we could observe a coincidental picture both in cases with good response and in cases with no response, although there were differences in the intensity of the response found in the two kinds of samples. In some cases smears show more evident cellular response than the corresponding biopsy, or vice versa. Contradictory findings were observed in only three cases. In four cases we could not get enough tissue for study.

Smears are much better than biopsies for observing reaction to irradiation, due to the fact that some biopsies do not show neoplastic tissue or even normal epithelium, especially when taken after radium therapy. Smears cover the complete cervical area; a biopsy only a small fraction of it, unless we take multiple samples.

On the other hand, in the biopsies taken eight weeks after treatment, it is possible to observe radio response, if it is present, more easily than in smears, because those changes are more evident in the basal cells which are not easily exfoliated.

A more complete study of tissue irradiation response may be done by combining these two complementary methods.

Bibliography

1. Graham, Ruth M.: Surg. Gynec. and Obst. 84:153, 1947.

JAMES A. MERRILL AND DAVID A. WOOD

San Francisco, California, U.S.A.

The response of carcinoma of the cervix to radiation therapy is being evaluated by studies of cytologic changes in vaginal smears and histologic changes in serial cervical biopsies with an attempt to correlate comparatively the data thereby obtained. Vaginal smears are obtained at weekly intervals during therapy. The cytologic study includes SR and serial RR counts based upon the criteria of Ruth Graham. (In most instances these are done by a cytotechnician who was sent to the Ruth Graham Laboratory in Roswell Park, New York, for the specific purpose of becoming thoroughly familiar with the criteria and techniques.) For the purpose of this report an RR value below 60% is considered a poor response and above 75% good. Intermediate values between 61 and 74% are discussed separately although considered as good. Serial biopsies are obtained from the growing edge of the tumor at weekly intervals, so far as possible, during therapy. These are evaluated histologically (Glücksmann) and cytochemically (Gusberg). (The cytochemical results will be mentioned briefly but will not constitute a significant portion of this discussion.) When repeated cervical biopsy showed absence of carcinoma and the cervical stroma showed a good radiation response, the case was considered with those classified as showing good histologic radiation response. Although as of this date the number of cases and duration of follow-up period is insufficient to permit accurate definitive evaluation, this preliminary report will attempt to correlate data obtained to date with clinical outcome.

The ease of obtaining vaginal smears constitutes a definite advantage of the cytologic as contrasted to the histologic technique in that a greater number of preparations can be obtained and the complication of bleeding obviated. The cervical biopsy specimens, however, have an advantage in that they do provide for additional histochemical studies, data from which may also be pertinent in the estimation of radiosensitivity. The greatest number of biopsies obtained from one patient before and during therapy was 12 but the average to date has been three to four. In many of the cases, one or more biopsies failed to reveal the tumor. Occasionally, vaginal bleeding has been precipitated. However, there has been no evidence that repeated biopsy has interfered with the healing process of the cervical lesion.

The usual radiation therapy consisted of two radium insertions (average total 5,300 mgh) separated by one or two weeks, followed by external x-ray (average 3,500 r tumor dose) by means of a 1,000 kv machine. A few patients were treated with the 70 mev Synchrotron (usually, 6000 rads calculated to the entire pelvis). Occasional cases received external radiation prior to radium therapy.

This is a report of our experience to date with 74 cases of carcinoma of the cervix; 48 cases with a follow-up duration period of one to two years and 26 cases, 6 to 12 months.

Patients with follow-up of one to two years. The major group consists of 48 cases treated during the calendar year 1957: three adenocarcinomas; one adenosquamous carcinoma; one primary squamous cell carcinoma of the vagina; and 43 squamous cell carcinomas of the cervix. One patient was treated by surgery and postoperative external radiation, one by a single radium insertion followed by Wertheim hysterectomy, and the remaining 46 by a combination of radium and x-ray. No therapy was altered on the basis of laboratory data.

The clinical staging of cases in this group is shown in the following tabulation:

	No. of Cases	Percent
Stage I	23	47.9
Stage II	19	39.6
Stage III	4	8.3
Stage IV	0	0.0
Stage 0	2	4.2
TOTAL	48	100.0

*From The Department of Obstetrics and Gynecology and The Cancer Research Institute, University of California, School of Medicine, San Francisco, California, U.S.A.

In this major group of 48 cases RR counts were done in 42 (and serial biopsy obtained from 36). The RR value was below 60% in 11 cases, 75% or greater in 24, and between 61 and 74% in seven. Of the 11 cases with low RR values, five are living and well (45%). In these five patients the lack of clinical correlation can be explained in four: one treated by cervicectomy and postoperative radiation only; one (carcinoma in situ), radiation; and two (Stage I) standard therapy with radium insertion followed by external radiation. The 5th patient had only one smear available for counting. Of the 24 cases with an RR value of 75% or greater, 18 are living and well (75%). Four of the seven cases with an RR value between 61 and 74% are also alive and well. A combination of these last two groups shows 31 cases (74%) with RR above 60%, of whom 22 are living and well (71%). Of the nine cases with recurrence, the lack of clinical correlation might be explained in five: four patients had advanced disease or incomplete therapy and one had a good local result despite persistence of the pelvic tumor. Thus far, the cytologic method of predicting response to radiation therapy has been of limited value in those cases with either very early or very late disease, the RR value frequently being the converse of the anticipated clinical outcome.

In the patients from whom cervical biopsies were obtained, histologic prognosis correlated extremely well with the clinical course and was, in general, more reliable than the cytologic evaluation - but less influenced by the stage of the disease. This advantage was particularly evident in evaluating cases with early disease. Of the 36 patients in the major group from whom cervical biopsies for study were obtained, the response in one was judged to be fair (good local response but followed by recurrence); good in 27 (75%); and poor in eight. Twenty-four of the 27 patients showing a good response are living and well. Of the three who died or developed recurrence, two had a good local result and one had a mesonephric adenocarcinoma of the cervix. Seven of the eight patients with poor histologic response are dead and one is alive with recurrent disease.

The frequency with which the findings from the cytologic and histologic methods agreed and could be correlated was an unexpected finding. Moreover, the value of both techniques combined appears greater than that of either technique alone, particularly in predicting an unfavorable response. In 31 cases both histology and cytology are available with positive correlation in 26. In 21 of these cases, where both techniques indicated a good response, 19 are alive and well (90%). Two have died: a late Stage III lesion being present in one patient and a Stage II in one who died after exenteration for local recurrence. The five patients who showed a poor response by both techniques either died or developed recurrent tumor. Five cases showed a lack of correlation. In each the histology indicated a poor response and the final outcome was unfavorable. Because the cytology seemed in error, the curves of the serial RR counts were reviewed. In two of these cases the curves were unmistakably good. Of the other, three patients, one refused further therapy (after receiving a small dose of external radiation); one showed a generally low RR curve with only a single high count of 63, and the remaining case had only a single vaginal smear available for counting. It is our impression, at present, that the general trend of the RR curve is important; and if low, the overall case should be considered as a poor response despite sporadic high counts.

Patients with follow-up of six to twelve months. Generally comparable results have also been obtained in the smaller group of 26 patients who have been followed for a period of only 6 to 12 months and all of whom were given primary radiation therapy. All had squamous cell carcinoma but one, who had an adenocarcinoma. The proportion of patients with advanced disease (clinical stages III and IV) is greater than that in the first group, 23.1 versus 8.3%. The clinical staging of cases in this group is shown in the following tabulation:

	No. of Cases	Percent
Stage I	13	50
Stage II	7	26.9
Stage III	4	15.4
Stage IV	2	7.7
TOTAL	26	100.0

Eight patients have died or have recurrent disease. Two of the deaths were unrelated to the tumor, and at autopsy no residual cancer was found.

RR counts were done on all 26 cases. Two had an RR value below 60%. Of these, one has recurrent disease and one is well after a Wertheim hysterectomy performed after the first radium insertion. There are 24 cases (92%) with RR above 60%, of whom 17 (71%) are living and well. Of the seven cases who are dead or have recurrent disease, five had far advanced lesions, one died of a skull fracture, and one showed no residual carcinoma at autopsy. Again the prognostic value of the cytologic method is found to be slight in those patients with far-advanced disease.

Good correlation was obtained in the histologic and cytochemical study of biopsy material from 23 cases, the results being similar in each instance. Of the three patients in whom the indicated response was poor, one has recurrent disease, 1 is alive (treated by Wertheim hysterectomy after radium insertion), and one is living and well. Twenty cases (87%) showed a good histologic response: 14 are living and well (70%), six are dead or have recurrent disease. The fact that five of these six cases were in clinical Stage III and IV would indicate that histologic evaluation is not so pertinent in the advanced case. These findings would also seem to indicate that laboratory prognosis in these advanced stages of disease is inferior to clinical evaluation and judgment.

In these 23 cases correlation between cytology and histology was similar to that obtained by comparison of histological and cytochemical techniques except for the one case living and well that was judged by the latter to show a poor response, but by the former "mixed," the RR value being 65% and the histologic response poor.

The over-all results from this small group of 26 patients are similar to those of the larger group with a longer period of follow-up.

Combined groups (long and short follow-up). Fifty-four of the 74 cases in the total series have been studied by both techniques (cytologic and histologic) with a positive correlation in 48 (88%), a poor response being judged in seven and a good response in 41. Six of the seven cases with poor response have either developed recurrent disease or died; the seventh is free of disease following a Wertheim hysterectomy. Of the 41 patients exhibiting good response, 33 are living and well (80%). In our opinion the deficiencies of these techniques as methods of predicting prognosis or response to radiation therapy are related to the selection of cases and the completeness or adequacy of radiation therapy. The ultimate value of such techniques depends upon the ability to select those patients who might respond better to surgery than to radiation therapy among a group who are indeed operable. Patients not suitable for evaluation by these techniques, particularly cytology, are those not treated by primary radiation therapy; those with very early disease with an excellent known prognosis by any mode of therapy, and those with far-advanced disease. Adjusting our series for these factors (specifically by excluding stage 0, microscopic Stage I diagnosed only by cone biopsy, Stages III and IV, cases with primary surgical treatment, those with inadequate radiation therapy, and those where death was due to causes unrelated to tumor and in which no residual carcinoma was demonstrable at autopsy) leaves a residual group of 39 patients. Cytologic and histologic evaluation showed a positive correlation in 35 (89.7%). Of these the response was poor in six and good in 29. In those with poor response the patients have either died or developed recurrent disease in contrast to those with good response, 27 of whom (93%) are living and well. Of the four cases in which positive correlation between the two techniques was absent, the clinical course was consistent with the histologic response in three patients and with the cytological response in one. Thus, in this adjusted group, those who received adequate therapy for clinical Stage I or II carcinoma showed radiation response that was usually similar by both techniques and that has correlated well with the clinical course to date. Of special interest and possible significance is the fact that in each case where the radiation response was judged poor by both techniques, the clinical outcome has been uniformly unfavorable. It is possible that this finding will prove to be of real value in the choice of therapy.

Additional information obtained during this study indicates that neither SR determination on vaginal smears prior to treatment nor histologic grading of the tumor has any significant value in predicting clinical radiation response. In regard to the latter, seven of eight patients with histologic Stage I carcinomas are living and well, 24 of 35 Grade II, and 20 of 28 Grade III. (In three of the 74 cases in the series the histologic grade was not determined.) These data are consistent with the findings of others that at present Grade I squamous cell carcinoma of the cervix continues to be encountered the least frequently.

SUMMARY

The combination of the cytologic and histologic methods in this pilot study has been encouraging in the attempt to evaluate the radiosensitivity of clinical Stage I and II carcinomas of the cervix.

Cytologic RR frequently indicates a poor response in patients with early cervical cancer who by other criteria have an excellent prognosis. Histologic response in this group appears to offer a more reliable index of probable radiosensitivity.

In patients with clinical Stage III and IV carcinoma of the cervix the study of exfoliated vaginal cells, and to a lesser extent of cervical biopsies, will frequently indicate a degree of radiation response greater than has been apparent by patient survival.

Despite the fact that one method is concerned with the response of normal cells and the other with the response of tumor cells to radiation, the results have been similar in the majority of cases. This suggests that tissue of the tumor and of the host show similarities of response to radiation.

Bibliography

1. Glücksmann, A.: *Brit. J. Radiol.* 14:187, 1941.
2. Glücksmann, A. and Way, S.: *J. Obst. & Gyn., Brit. Emp.* 55:573, 1948.
3. Graham, J.B., and Graham, R.M.: *Ann. New York Acad. Sc.* 63:1458, 1956.
4. Graham, R.M.; *Surg., Gynec. & Obst.* 84:153, 1947; II. Prognostic significance, *ibid.* 84:166, 1947.
5. Graham, R.M., and Graham, J.B.: *Cancer* 8:71, 1955.
6. Gusberg, S.B.: *Am. J. Obst. & Gtn.* 72:804, 1956.
7. Gusberg, S.B., Long, M., and Hill, J.C.: *Ann. New York Acad.* 63:1447, 1956.
8. Merrill, James A.: *Progress in Radiation Therapy.* New York, 1958, Grune and Stratton.

DISCUSSION

CONSTANTIN HEROVICI, Villejuif, Seine, France:

The report of Merrill and Wood, whose procedure of histoprogno- sis of carcinoma of the uterine cervix is very similar to the one we practice in our institute, causes some remarks: I would be very curious to learn the criteria on which they base their diagnosis of good radiation response of the connective tissue during therapy, since we have never found either a marked fibrinoid necrosis or an inflammatory reaction of the tumor stroma during irradiation.

It is only one month after cessation of treatment that the maturation of the collagen permits one to draw conclusions as to the prognosis. Also, when they look for histochemical criteria in histological sections, why don't they do the same in vaginal smears? In biopsies taken during irradiation treatment it is especially not infrequent that there is no normal cervical epithelium, whereas the vaginal smear always contains normal benign cervical cells. It is these cells that show an increase of RNA preceding a decrease of nuclear DNA and thereby permits one to establish a cytoprogno- sis. This has been found valid in 85% of a series of 117 cases of carcinoma of the uterine cervix, followed for three years after treatment since 1954. It seems to us that a lapse of two years is insufficient for a prognostic study, particularly when the verification of a favorable prognosis is concerned.

When the authors in a series of 36 patients with carcinoma of the uterine cervix found 75% with a good radiation response and 20% with a poor response, they must have combined the good as well as the medium responses into the first category. We could not find more than 60% good radiation responses, 15% medium responses and 20% poor responses, (i. e., absence of any radiation reaction) in a series of 300 patients with carcinoma of the cervix. This series was composed of 95% more or less differentiated squamous carcinomas, 5% pure or mixed adenocarcinomas and two sarcomas. Likewise, it would be interesting to know whether or not the authors have separated the irradiation changes observed at the site of the tumor itself from those in the normal benign epithelium and connective tissue. If so, we would like to know whether or not the authors prefer to study the radiation response on the tumor itself or on the benign tissues.

We completely agree with the authors that very little prognostic value is attached to the pre- therapeutic vaginal smear and biopsy, since we were not able to find any significant correlation between the histomorphological type of the tumor and its radio-sensitivity. At this point I would like to emphasize the type of morphological and histochemical stroma reaction which does seem to permit prognostic appreciations, regarding the radio-sensitivity as well as the development potentialities of the uterine carcinomas.

CLOSING REMARKS

JAMES A. MERRILL and DAVID WOOD:

This report concerns preliminary observations on histologic and cytologic techniques for deter- mining radiation response. Certainly, the follow-up is too short for a valuable prognostic study. In this we completely agree with Herovici. However, the histologic technique, especially, has been effective in pre- dicting an unfavorable response, which does not need a long follow-up, and in those cases where radiation response was judged poor by both techniques, the clinical outcome has been uniformly unfavorable.

Seventy-four percent good RR is somewhat higher than that reported by other investigators. We have considered as good an RR above 60%, and this does include cases which might be considered a "medium" response by others.

The two techniques differ significantly in that the cytologic method is concerned only with the morphologic effect of radiation upon normal vaginal cells. We have made no attempt to study the nucleic acids in these cells. The histologic technique is concerned primarily with the study of changes in the malignant cells. We have not studied the changes in the benign epithelium in these biopsies, although this has been done by Glücksmann. Indeed, the cervical biopsies frequently contain no normal cervical epithe- lium. A conscious effort is made to include only tumor tissue. However, the changes in the stroma are taken into account in the final determination of radiation response. Following radium therapy, we note the stromal changes described by Warren. The biopsies which show a good response to radium therapy are characterized by hyalinization and fibrosis of the connective tissue, telangiectasia and thickening of the arteriolar walls. Necrosis may also be present. A frequent accompaniment is an acute inflammatory in- filtrate. However, it is impossible to determine if this is a response to radiation or to the malignant tumor tissue. It is quite true that these stromal changes occur very slowly following external x-ray therapy and may be apparent only upon completion of treatment. However, stromal changes are quite frequent following radium application.

We are impressed with the frequency with which the radiation response of normal vaginal tissues parallels the radiation response of the tumor in biopsy specimens, despite the fact that one is said to measure host-response and the other tumor-response. Also important to us is the observation of the in- adequacy of either technique in evaluating patients with far-advanced disease.

DOES IRRADIATION INFLUENCE THE KARYOPYKNOTIC INDEX?

OLLE KJELLGREN

Gothenburg, Sweden

We have studied the relationship between cell changes and hormonal effect as reflected by the cell composition of the vaginal smear. Hormonal effect was then judged on the basis of (a) the Karyopyknotic, Eosinophilic and Basal Cell Indices and (b) grading by hormonal levels (see "The Clinical Factors Associated with Good Cytological Response to Irradiation" in this Symposium).

The Karyopyknotic Index was taken to be the ratio of superficial cells showing karyopyknosis to all squamous cells in the smear, expressed in per cent. The nucleus of a superficial cell was considered pyknotic if it was condensed and no chromatin structure could be distinguished under a 600 power microscope. The Eosinophilic and Basal Cell Indices were the corresponding ratios of eosinophilic and basal cells respectively to all squamous cells.

Plots of the mean Karyopyknotic and Basal Cell Indices interpolated for each day after the first radium application in the poor and good response groups are shown in Figures 1 and 2. The results were the following:

Starting from a low value (of the order of a few per cent) at the first radium application, the mean Basal Cell Index for the poor response group increased significantly up to the commencement of x-ray irradiation some seven or eight weeks later. The relationship between the rising Basal Cell Index and time satisfies the regression equation (Fig. 1):

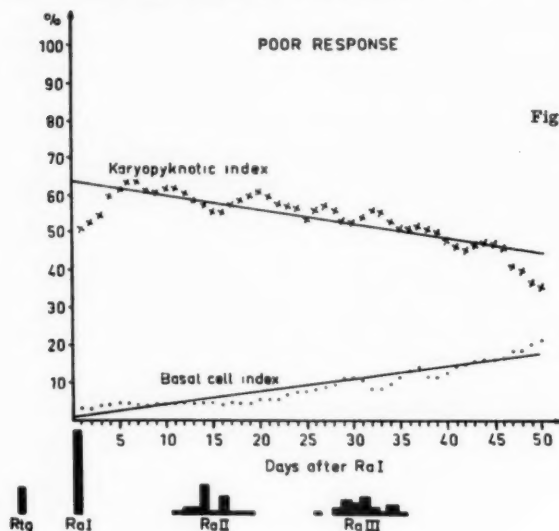


Fig. 1. Mean daily Karyopyknotic Index and Basal Cell Index in the poor response group. The histograms represent the number of patients receiving treatment at a particular time.

$$Y = 0.73 \pm 0.333 x;$$

$$(S_b = 0.016, df = 48, t = 21.3, p < 0.001).$$

In the good response group the starting point at Ra I of the curve for the mean Basal Cell Index lies at a higher level. The curve then rises significantly with time and has the regression equation (Fig. 2):

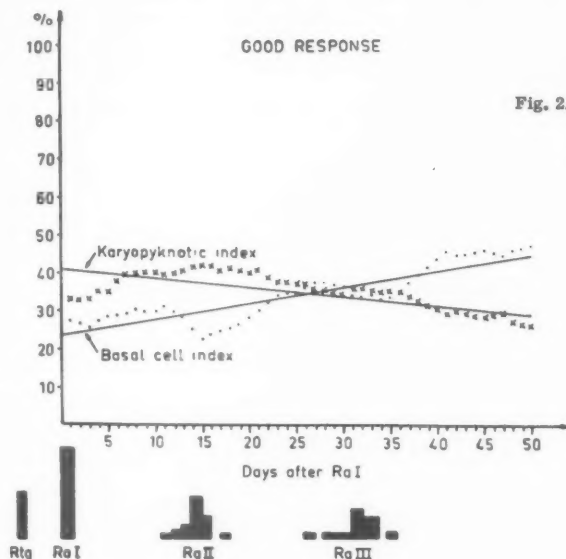


Fig. 2. Mean daily Karyopyknotic Index and Basal Cell Index in the good response group. The histograms represent the number of patients receiving treatment at a particular time.

$$Y = 23.44 \pm 0.432 x;$$

$$(S_b = 0.032, df = 48, t = 13.5, p < 0.001).$$

The curves representing the mean Basal Cell Index with time as parameter show that the rate of increase was higher in the good than in the poor response group, the regression coefficients differing significantly ($b_{\text{good}} - b_{\text{poor}} = 0.099$, $S_{b \text{ diff}} = 0.036$, $df = 96$, $t = 2.78$, $0.01 > p > 0.001$).

The curve for the daily mean Karyopyknotic Index in the poor response group slopes significantly downwards with time and has the regression equation (see Fig. 1):

$$Y = 63.61 - 0.394 x;$$

$$(S_b = 0.038, df = 48, t = 10.4, p < 0.001).$$

In the good response group, similarly, the curve for the mean Karyopyknotic Index slopes significantly downwards, but the entire curve is transposed to a lower level. The regression line has the equation (Fig. 2):

$$Y = 41.21 - 0.228 x;$$

$$(S_b = 0.031, df = 48, t = 7.3, p < 0.001).$$

It appears that the rate of decrease of the mean Karyopyknotic Index in the good response group significantly exceeded that in the poor response group ($b_{\text{good}} - b_{\text{poor}} = 0.166$, $S_{b \text{ diff}} = 0.049$, $df = 96$, $t = 3.39$, $0.01 > p > 0.001$).

Accordingly, in the good response group the Basal Cell Index lay on a higher over-all level and increased more rapidly, while the Karyopyknotic Index lay on a lower over-all level and decreased more rapidly than in the poor response group. This implies that, as reflected by the cellular composition of the vaginal smear, the estrogen stimulation was less intense and subsided more rapidly during radiation therapy in the good response group than in the poor response group.

Bibliography

1. Kjellgren, O.: Acta Radiol. Suppl. 168, 1958.

ANDRÉE PELZER

Liege, Belgium

As the vaginal epithelium responds to hormonal stimulation, it seems logical to think that if the ovaries are irradiated with a certain dose sufficient to inhibit the development of the Graafian follicle, a decrease in the Karyopyknotic Index (K. I.) will appear. This is evidently shown by our material, a group of 25 women, all before menopause. All of them presented cervical carcinoma in various stages; I (17 cases), II (5 cases), III (3 cases). As a general rule, 1600 r were given toward the parametric by two anterior and two posterior portals. During the same period of time, radium was further given in three applications, each time in the endocervix and in the vagina against the cervix, to the total amount of 6800 mgh. This radiotherapy was completed in about 25 days.

Smears were taken prior to irradiation and not later than one month after its completion. The results are shown below:

KARYOPYKNOTIC INDEX (in %)	Number of cases	
	before irradiation	after irradiation
0 - 10	1	12
11 - 20	4	9
21 - 30	5	1
31 - 40	0	1
41 - 50	5	1
51 - 60	4	1
61 - 70	4	0
71 - 80	0	0
81 - 90	2	0

Thus the decrease of the K. I. is marked: in 21 out of the 25 cases it is below 20% after irradiation compared with the five cases prior to irradiation. In only two cases it remains over 40%, while it was present in 5 cases before irradiation.

Such a decrease also appeared very soon after irradiation in smears taken not longer than one month after its completion.

It should be mentioned that none of these women menstruated after completion of irradiation.

I believe that it can be concluded that:

1. Irradiation induces a decrease of the K. I.
2. If the total dose of x-ray is a relatively high one, as in our cases, the decrease of the K. I. is marked and occurs very soon after irradiation.

The following questions deserve further investigations:

1. What is the minimum dose of x-rays able to influence the K. I. ?
2. When using small doses of x-rays such as for castration, how long is the latent period before changes in the K. I. appear?
3. How long does it take before the K. I. becomes stable again at its new level? All my patients were submitted to surgery and could not be followed further in that point of view.
4. Do very small doses of x-rays, as used for diagnostic purposes, influence the K. I. ?
5. Does irradiation influence the K. I. in menopausal women? In a group of 20 women irradiated in the same way as described, the period of time between menopause and irradiation was from 5 to 32 years, between irradiation and the cell count, from 2 to 15 years. In 14 of them, the K. I. was between 0 and 10%, in five between 11 and 20%, in one between 21 and 30%.

GUILLERMO TERZANO

Buenos Aires, Argentina

Patients receiving radiation as treatment for uterine carcinoma have been carefully followed by one of our associates (Arturo A. Arrighi) with serial vaginal smears for the study of radiation response (RR). A record of the percentages of each type of benign squamous vaginal cells has also been kept.

Regarding the Karyopyknotic Index before and after radiation, patients may be classified into two main groups:

- I: Those who received radiation before menopause (normal vaginal smears prior to treatment).
- II: Those who received radiation after menopause (vaginal smears showing low or absence of estrogenic level prior to treatment).

I. In the first group (women before menopause) slight regressive changes in the vaginal epithelium were usually noticed as an early effect of castration. About the second or the third week after radiation the Karyopyknotic Index began to return to the level observed before treatment; it may even be higher than prior to radiation in the fourth to the sixth week. About the tenth to the twelfth week most of the smears returned to the atrophic type. This happened, according to our records, in 14 (63.6%) of 22 patients. In the other eight cases, six (27.2%) smears remained atrophic and 2 (9.09%) exhibited no noticeable changes.

II. We have observed in the second group (women after menopause):

A. Those with hypotrophic vaginal smears (low estrogenic level): in 15 (71.4%) of 21 women, no remarkable changes in the percentage of the superficial (karyopyknotic) cells, intermediate cells and parabasal cells. In four (19%) atrophic changes were noticed, and in two (9.5%) some increase of the Karyopyknotic Index.

B. The group of 56 women with atrophic vaginal smears (estrogenic level absent): 36 (64.3%) showed an increase of the superficial (karyopyknotic) cells occurring between the first and the third week after treatment, which began to decline two to five weeks later; 20 (35.7%) smears remained of the atrophic type.

It is our opinion that irradiation may influence to a certain extent the Karyopyknotic Index in vaginal smears.

DISCUSSION

LUIS MONTALVO-RUIZ, Madrid, Spain:

For the study of the Karyopyknotic Index one divides patients into two groups: 1) those with high estrogenic activity and 2) those with low or no estrogenic activity. We form these groups regardless of whether or not the patients are postmenopausal, since we know that 32% of postmenopausal women have good estrogenic activity and a high Karyopyknotic Index. We believe that the Karyopyknotic Index is higher in patients with carcinoma before and after the menopause. This is confirmed by the works of Dearing and Winifred (1) and Erica Wachtel (3).

In the first group of patients our findings resemble those of Terzano's (a group of 52 women) with the exception of our case number nine who had a low estrogenic activity before radiation. This patient's Karyopyknotic Index increased during the following weeks. Three and one half months after radiation it was very high.

There was response to radiation with increase of the Karyopyknotic Index in 11 of the 43 patients with a low or an absence of estrogenic activity. In our case number 11, the karyopyknotic cells appeared two months after radiation. In our case number 27 the karyopyknotic cells appeared 37 days post radiation, and in case number 66, 30 days after radiation.

We conclude that variation of the Karyopyknotic Index after radiation depends basically upon the hormonal status of the patient, and that the variations are extremely irregular.

Bibliography

1. Dearing, R. and Winifred, L.: J. Obst. and Gyn. Brit. Emp. 63:375, 1956.
2. Montalvo-Ruiz, L.: Acta Gin. 8:439, 1957.
3. Wachtel, E.: Triangulo Rev. Sandoz 3:281, 1958.

OTAKAR NYKLÍČEK, Náchod, Czechoslovakia:

My own experiences agree with the observations of Terzano. I would like to point out that somewhat higher levels of the Karyopyknotic Index were found in postmenopausal women with cervical carcinoma

than in healthy menopausal ones. This is, of course, known from former studies (Ayre, Erica Wachtel). We observed that after radiation therapy with a full cancer lethal dosage, it took at least three to four months before the smear with high Karyopyknotic Index would change into an atrophic type of smear, i. e., the so-called "shift to the left" occurred in the vaginal cytogram, as we call the appearance of cells from the lower layer of the vaginal epithelium.

We draw attention to the significant diagnostic support the vaginal smears offer in recurrences of carcinomas. On the contrary, there is a substantial "shift to the right," i. e., the occurrence of cells from the upper layers of the vaginal epithelium and the increase of the Karyopyknotic Index, which forms an alarming symptom.

CLOSING REMARKS

GUILLERMO TERZANO:

Though we cannot prove it accurately, it seems correct to state that in the series of Montalvo Ruiz, Nyklíček and ours, the Karyopyknotic Index in vaginal smears has been influenced to a certain extent by irradiation.

In order to answer the question properly, we refer only to those changes possibly due to irradiation, because we consider that for a prognosis a high Karyopyknotic Index long after treatment, is not the same as the direct influence of irradiation upon the vaginal epithelium.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

RECURRENT CARCINOMA AND THE PRESENCE OF RADIATION CELL CHANGES

ARTURO ANGEL ARRIGHI

Buenos Aires, Argentina

The presence of malignant cells in a post-treatment smear is considered as evidence of recurrent carcinoma only when the cancer cells that have been observed before the application of radium has disappeared and there has elapsed at least two or three months from the time the radium was removed.

During this time the morphological changes induced by radiation in the normal squamous cells are attenuated or have disappeared and the differential diagnosis between the altered non-malignant cells and the cancer cells is not too difficult.

In order to make the diagnosis of malignancy with relative accuracy, the most valuable sign to be considered is the presence of an abnormal nuclear-cytoplasmic ratio, whereas normal irradiated cells exhibit a normal nuclear-cytoplasmic ratio.

EMMERICH von HAAM AND RICHARD ALBERY

Columbus, Ohio, U.S.A.

According to Zimmer (1), the cytologic diagnosis of recurrent malignant tumors of the uterine cervix after irradiation therapy is not difficult whenever we deal with cells which show the typical criteria of malignancy. The material upon which our study is based consists of 77 cases which recurred from 3-37 months after completion of radiation therapy and which were proven by subsequent biopsy. Most of the patients were followed in our Cancer Clinic at intervals of 1-3 months. Biopsies were performed after the appearance of suspicious cells in the vaginal smears or after clinical evidence suggested a recurrence. In only two cases were we unable to find malignant cells in the smear in spite of clinical recurrence and positive biopsy. Fifty-three cases thus far have died from the disease. Clinical data, which will be published elsewhere, show some correlation between the extent of the disease and the time and frequency of recurrence. No correlation could be found between the cytology of histopathology of the tumors, the type of treatment and the frequency or period of recurrence. Carcinomas of clinical Stages III and IV recurred three times more frequently than carcinoma of the clinical Stages I or II.

The Ohio State University radiation treatment consisted usually of intravaginal application of cobalt 60 in the form of surface application by colpostat or tandem, or cobalt 60 incorporated in needles or threads. This was followed up by external radiation with a 225 kilowatt x-ray unit. Of the 77 patients with recurrent carcinoma, 21 had a well differentiated type of squamous cell carcinoma, 38 had tumors classified as histological grades two or three, while 18 suffered from highly anaplastic tumors, which according to Glücksmann (2) give the poorest prognosis as far as radiation therapy is concerned. Vaginal smears were taken before commencement of therapy, at daily and weekly intervals during the therapy and at intervals of 1-3 months when the patients were followed in the Cancer Clinic. The smears were obtained directly from the exposed suspected lesions, or if this was not possible, from aspiration of the vaginal pool. In the examination of the smears we followed carefully any evidence of radiation injury to normal or malignant cells and studied the types of inflammatory reaction accompanying these changes (3). It has been our experience that undamaged malignant cells disappear usually during the first three weeks following the treatment with cobalt 60 and are replaced by malignant cells showing vacuolization of the cytoplasm and/or nuclear changes ranging from vacuolization to pyknosis, karyorrhexis or karyolysis. This type of cell change may be found up to eight weeks after treatment and is accompanied by a severe inflammatory reaction which is either of the diffuse type or shows pronounced agglutination of leukocytes around clumps of necrotic cells. After a

period of 3-6 months these changes, classified as acute radiation changes, give way to the chronic radiation changes which may persist in some form or other throughout the remaining life of the patient. This consists of the appearance of severe radiation dyskaryosis among benign cells and a cellular reaction of the histiocytic type with giant cells and large radiation fibroblasts. In the healing lesions all necrotic debris disappears from the cytological smear and the patient can be considered clinically, as well as cytologically, cured. In the cases of unsuccessful radiation therapy this cytological picture of Mohr's Finalreaktion (4) is never established, and degenerated malignant cells never disappear completely from the cytological smear in spite of negative biopsies. In those cases we cannot speak of tumor recurrence but must assume that portions of the tumor continue to proliferate in spite of radiation therapy and that the biopsy was too superficial to demonstrate the remaining viable tumor tissue.

In order to diagnose true tumor recurrence we must establish clinically as well as histologically or cytologically as accurately as possible that all local tumor tissue was successfully destroyed by the treatment only to reappear after a certain period. The reappearance of tumor cells in a patient apparently successfully treated may assume two different aspects: We may observe, as did Zimmer, malignant cells showing unmistakable changes compatible with radiation changes. Those cells are rather grotesque, usually very large and easily recognized (Fig. 1). They appear first in small numbers and a biopsy is often negative. In the course of several months they slowly increase in number and the biopsy finally becomes positive. The patients usually show early clinical signs of progressive disease. In the other type of tumor recurrence we find small undamaged malignant cells appearing in quickly increasing numbers which usually belong to the undifferentiated type regardless of the previous histological type of tumor (Fig. 2).

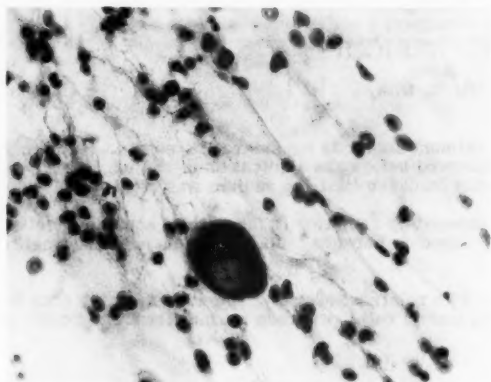


Fig. 1. Malignant cell appearing one year after successful radiation therapy showing characteristic radiation changes. (450X)

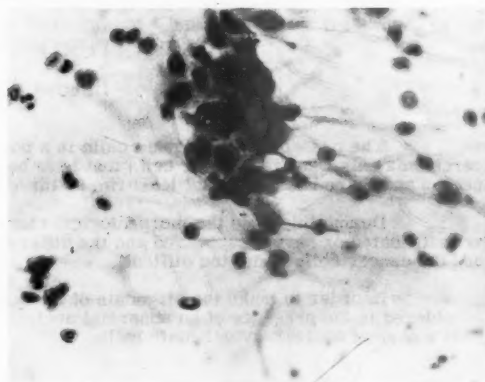


Fig. 2. Clump of small undifferentiated malignant cells appearing four months after radiation therapy to a well differentiated carcinoma. (450X)

The recurrence can often be demonstrated clinically in the form of a small nodule or a pink bleeding lesion in the vicinity of the previous lesion, and the biopsy becomes quickly positive. Glücksmann states that an increase in cell differentiation is one of the major factors in successful radiation therapy of individual cancers. The recurrence of carcinoma of the cervix in the form of anaplastic tumors therefore should carry an especially poor prognosis for our patients. The truth of this statement may be borne out by the fact that all 12 cases which had a recurrence of the tumor in highly anaplastic form, with small viable tumor cells without radiation changes, died within one year after appearance of the recurrence.

Bibliography

1. Zimmer, T.S.: Transac. Fifth Ann. Meeting, Inter-So. Cytology Council 5:89, 1957.
2. Glücksmann, A.: Transac. First Internat. Cancer Cytology Congress 1:307, 1956.
3. Von Haam, E.: Am. J. Clin. Path. 24:652, 1954.
4. Mohr, H.: Gynäkologischen Cytodiagnostik (H. Smolka and H. J. Soost). Stuttgart, 1956, Georg Thieme.

B. CORNELIS HOPMAN

Miami, Florida, U.S.A.

The recurrence of carcinoma cells in a cytologic slide of a patient treated with radiation for a cervical carcinoma gives the patient an unfavorable prognosis. It is accompanied by vanishing of the

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radiation cell changes in most of the normal and cancer cells. In such a case, however, it is not always easy to diagnose cells as real cancer cells. Sometimes they show coarse clumping, a prominent nucleus, hyperchromasia and sufficient difference in size and shape to regard them as highly suspicious. Yet their regularity in chromatin division and similarity in general cell appearance makes their appearance less ominous. It is far more difficult to diagnose carcinoma in radiated than non-radiated cells.

Radiation itself changes the cells. As pointed out by Ruth Graham (1) the specific cellular changes which occur in benign squamous cells during radiation therapy are: 1) an increase in both nuclear and cellular size, 2) vacuolization of the cytoplasm, 3) multiple nuclei, 4) wrinkling of the nucleus. In addition, we found in cancer cells changes such as alterations of the nuclei which showed lessened density of the chromatin and loss of coarse clumping. We noted an increase in histiocytes of the foreign body giant cell variety with multiple peripheral nuclei and infiltration of cells with leukocytes, in some instances completely covering the cells.

Many of these changes, however, occur in normal as well as in cancer cells, complicating the cell picture of radiated patients and requiring great experience to differentiate the "good" from the "bad" cells. "If I don't advise the cytologist that radiation has taken place, I very often get a positive cancer diagnosis in my patients radiated for cancer of the cervix and showing a favorable course," a gynecologist told me. A cytologist therefore may be fairly sure of his positive cancer diagnosis in non-radiated patients, but he must be very reserved in his diagnosis when radiation has taken place. More experience in the course of the years has increased our knowledge and I think in most cases we are now able to diagnose a recurrence. The cancer cells show the same known characteristics: Hyperchromasia, enlarged nucleolus, difference in nuclear size and form and chromatin division.

Very often there is a prediction of the things to come in the follow-up of the slides which the cytologist examines. The vaginal epithelia become less numerous and more eosinophilic: SR declines. Infection increases, measured as a ratio of growing numbers of leukocytes and decreasing numbers of squamous cells. The cells become more dyskaryotic and at last, amidst increased infection, cancer cells are found which without any doubt make the diagnosis of a recurrence evident. Reading some thousands of slides of radiated patients we have come to the conclusion that infection plays an important part in the etiology and prognosis of cervical cancer. Facts that support the infection theory are numerous and powerful (Fig. 1).

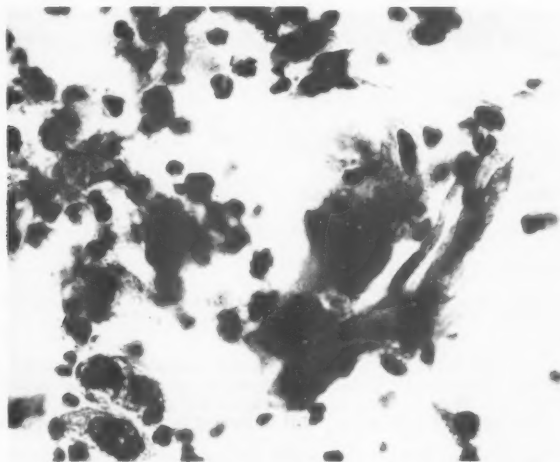


Fig. 1. Recurrence of radiated cervical cancer. Cancer cells amidst increasing infection and eosinophilia.

Cashman (2) studied the role of deep cauterization in the prevention of carcinoma of the cervix and found that in a series of 10,000 treated women only two cases were known to have occurred. In a study of the cause of death of 13,000 nuns in a convent in Quebec covering 20 years, cervical carcinoma was never listed. This one fact becomes even more startling when it is noted that carcinoma of the cavum uteri appeared as the cause of death in 12 of these women. The frequency of cervical carcinoma is seven times that of endometrial carcinoma which would mean that approximately 84 cervical cancers were to have been expected in this group (3). Prominent inflammatory cell concentrations at the squamo-columnar junction were found in 24% primigravida and in 60% multiparous women. Cervical carcinoma has been shown to be 16 times more frequent in multiparous women (4). In histologic slides, infection usually is found in the malignant portion of the tissue, whereas the normal tissue is free of any infection (Figs. 2, 3).

Consideration of infection as an important factor in the cancerous process also clarifies the failure of SR to predict the prognosis in so many cases. There is only a cure rate of approximately 70% in the good response group, because the cervical smears can only display a local inflammatory reaction of that tissue where the smear was actually obtained. It can never give an answer to infections in tissues remote from the local smear site, so that cancer can spread in parametrium and pelvic lymph nodes to a great extent when the cervical smear still gives a favorable SR response. The reverse is also true, when the patient is apparently in good condition, notwithstanding severe local infection.



Fig. 2. Increasing inflammatory infiltration of the subepithelial tissue from the normal (right) epithelium to the basal cell hyperplasia and carcinoma in situ (left).

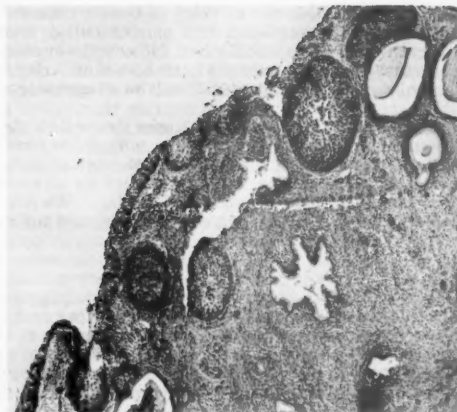


Fig. 3. Same histological slide as Fig. 2, further to the left: Increasing inflammatory infiltration in the malignant tissue.

CONCLUSION

The presence of radiation cell changes of normal as well as cancer cells has vanished in most of the vaginal cells when recurrence of the cancerous process takes place. This is accompanied by increasing eosinophilia of the vaginal epithelia as well as infection, measured by the ratio of increasing numbers of leukocytes and decreasing numbers of vaginal epithelia. SR gives information only of the condition of that tissue where the smear is actually obtained.

Bibliography

1. Graham, R. M.: Transac. Sec. Ann. Meeting Inter-Soc. Cytology Council 2:63, 1954.
2. Cashman, B. Z.: Am. J. Obst. & Gyn. 41:216, 49:190, 1941.
3. Gagnon, F.: Am. J. Obst. & Gyn. 60:516, 1950.
4. Murphy, E. J. and Herbut, P. A.: Am. J. Obst. & Gyn. 59:384, 1950.

LEOPOLD G. KOSS
New York, New York, U.S.A.

In our experience the presence of cancer cells for a period of four weeks following the completion of radiation treatment for cervical cancer carries an ominous prognosis. This observation applies whether or not the cancer cells show radiation effect. Only in a very few exceptional cases did the presence of cancer cells for as long as six weeks following completion of treatment, fail to result in clinical recurrence.

Recurrent carcinoma, in patients who were apparently successfully treated and free of disease for six months or longer, may be readily detected on cytological grounds if the tumor is present anywhere within the cervix or vagina. The cancer cells shedding from the foci of recurrent carcinoma are, as a rule, free of any radiation changes and may be readily differentiated from benign epithelial cells showing late radiation effects. Of interest is the fact that recurrent epidermoid cancer of the cervix not infrequently presents itself as a foci of in-situ carcinoma.

DISCUSSION

ARTURO ANGEL ARRIGHI:

In our material we have observed only seven cases with malignant cells in smears obtained at the end of roentgen therapy (three to four months after the radium application was removed). All these women died of invasive cancer, which was unaffected by the therapy.

B. CORNELIS HOPMAN:

I agree with Koss that the presence or recurrence of cancer cells for four to six weeks following the completion of radiation treatment carries an unfavorable prognosis. It is mostly followed by a clinical recurrence. If the cancer cells are free of radiation changes, the differentiation of benign from malignant epithelium may be easily made. Difficulties may occur in the recognition of radiated benign cells and malignant cells and great experience may be needed to make the differentiation. I believe that hyperchromatism, irregularly divided chromatin and enlarged nucleoli are the most important criteria.

Drs. von Haam and Ceelen give us a clear insight into the exact cytologic prognosis of recurrent radiated cancer. Of 77 cases recurring from three to 37 months, 53 have died, thus far showing the importance of cytologic follow-up of radiated cervical cancer. According to Herbert E. Schmitz the chances of survival following treatment of a Stage I carcinoma are better than twice those of Stage II and eight times better than those of Stage IV. I fully agree that the presence of undifferentiated cells is an even more unfavorable sign and indicates an advanced cancer. This applies before radiation as well as after radiation. They are small cells usually with only a small rim of cytoplasm. The nucleus displays the known cancerous characteristics, but by its small size it is easily overlooked. The slide very often contains a great deal of blood and many leukocytes. The number of these anaplastic cells is often minimal, not more than a few in a microscopic field, making comparisons and looking for variations in shape and size still more difficult. I agree that the appearance of histiocytes after radiation and disappearance of the necrotic debris are favorable signs, but I regard the inflammatory reactions in the slides apparently of more importance than von Haam does. In reading 50 slides a week of radiated patients during the past four years I have come to the conclusion that infection is of considerable importance in etiology and prognosis of cervical cancer.

I consider, as does Arrighi, the presence of an abnormal nuclear-cytoplasmic ratio of great importance in the diagnosis of malignancy, especially if this is combined with hyperchromatism and coarse clumping of the chromatin.

LEOPOLD G. KOSS:

I was very much interested in the photographs submitted by Hopman which represent an in-situ cancer with extension to the endocervical glands.

This is quite in keeping with our experience mentioned briefly in my contribution that recurrent cervical cancer after radiation frequently assumes this form.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

ARE THE DEGREE AND THE DURATION OF RADIATION CELL CHANGES DEPENDENT UPON THE DOSAGE OF IRRADIATION OR THE PERIOD OF TIME OVER WHICH IT IS GIVEN?

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

The degree of radiation cell changes is directly related to the dosage of x-radiation. In the patient who is being treated primarily by x-radiation the response can be correlated directly to the amount of radiation reaching the cervix. To speak of percentage of radiation response during x-ray treatment without relating it to the amount of radiation is similar to trying to plot a point on a graph knowing only one factor. Figure 1 shows a good response to radiation. The percentage response is indicated for 1000r, 2000r, 3000r and 4000r at the cervix. The dotted horizontal line indicates the level of radiation response which divides the good and poor response groups at that particular amount of radiation at the cervix. These are 25% at 1000r, 50% at 2000r, 70% at 3000r, and 75% at 4000r. The curve in Figure 1 indicates that this patient had a response of 65% at 2000r. This is in the good response range at 2000r but would be in the poor response range if the dose at the cervix were 3000r or 4000r. Thus, for interpretation of the percentage of radiation response it is absolutely essential to know the dose at the cervix, if the primary treatment is x-ray. However, that there are other factors than dosage operating may be seen by comparing Figure 2 with Figure 1. The amount of radiation delivered is identical. The clinical stage is the same. Yet one patient responds beautifully to the radiation, the other poorly. The first patient is well without evidence of disease. The second patient succumbed to her disease.

If, on the other hand, the primary treatment is by radium application rather than by x-ray, it is not possible to use dosage since the entire amount of radiation is delivered in a relatively short period of time. It takes time for radiation changes to develop and reach their peak after radium application. The cytologic response must be interpreted in relation to the lapse of time since the radium was applied. This is essential. Figure 3 illustrates this point. A smear taken five days after radium application showed a percentage response of 67 in the poor response range. This is too early to try to judge the response accurately. This cellular response to radium is a temporary one and falls off rather rapidly in some instances. The smear on the twenty-first day showed only 60% response - again in the poor range. Of course between these two smears the curve has risen to well over 70%, and the response is entirely adequate.

Figure 4 illustrates a poor response to radiation. Seeing the entire curve, it is a fairly obvious poor response. But let us consider the smear on the twentieth day. There was 58% radiation response present. This is almost identical to the count of 60% on the twenty-first day of the patient who had a good response to radiation. If we had had only these two smears, it would have been impossible to distinguish between these two cases. Yet they are quite different. The patient who had a satisfactory response to radiation is well and free of disease 4-1/2 years later. While the patient whose cytologic response is unsatisfactory died within two years of treatment.

In interpreting the cytologic response to radiation it is preferable to have an entire curve, since patients vary considerably in the speed of their response. If it is impossible to have an entire curve of response smears taken, then those obtained between the ninth and sixteenth day are usually adequate. If only one smear is taken, then the fourteenth day is preferable, counting the day of radium application as day one. It should be stressed that smears taken before the ninth or after the sixteenth day are of no value from a prognostic standpoint, if the percentage of response is below 70%. It is impossible to know whether the curve went up and then fell off or whether it ever went up at all. The time factor is critical for proper interpretation.

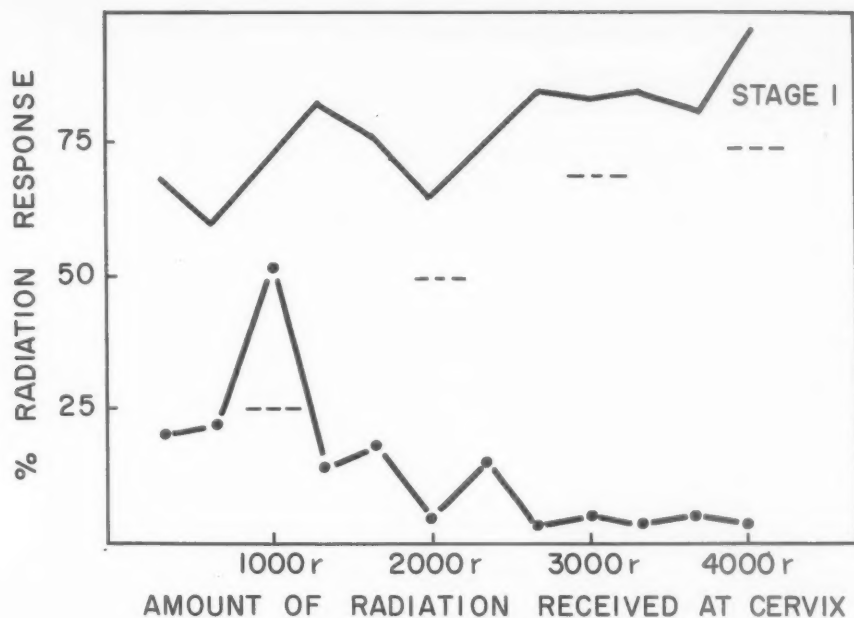


Fig. 1

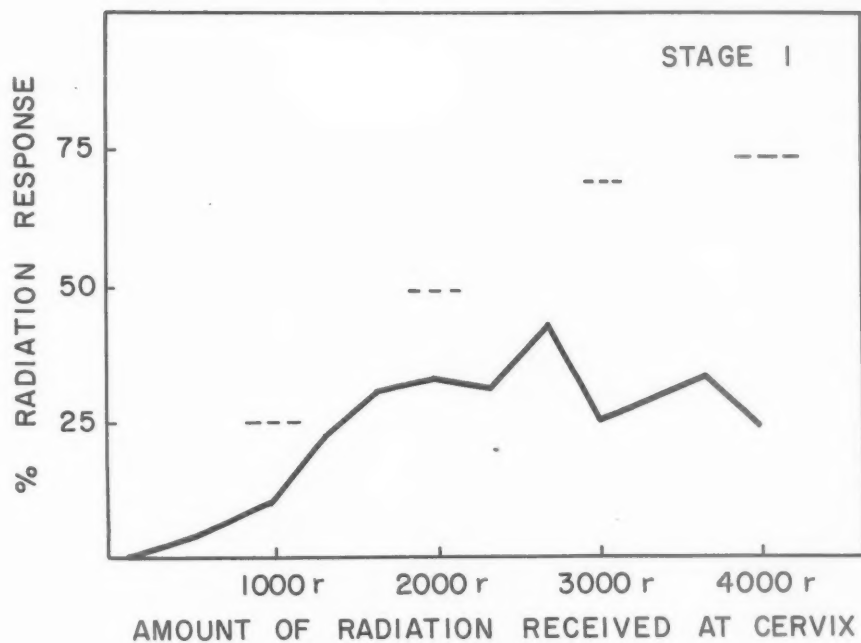


Fig. 2

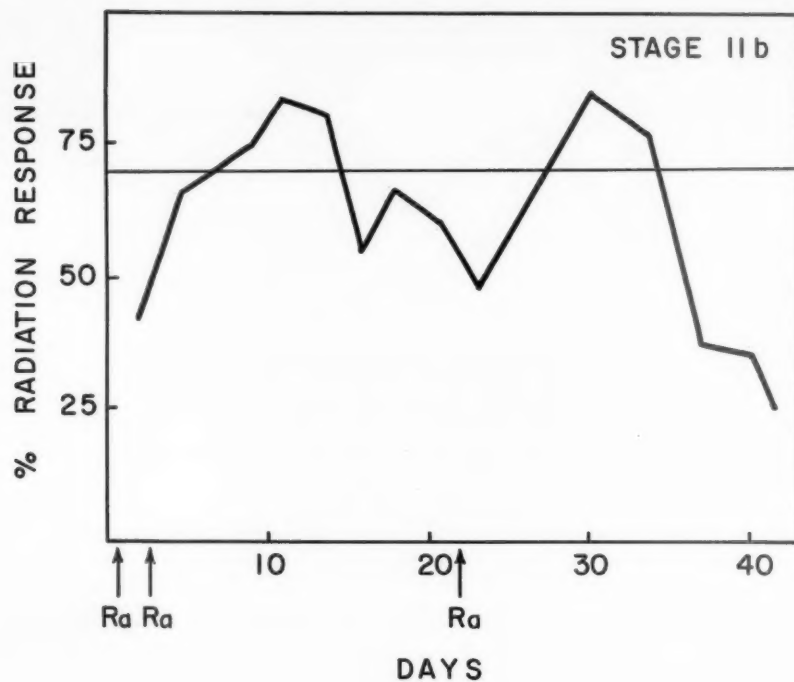


Fig. 3

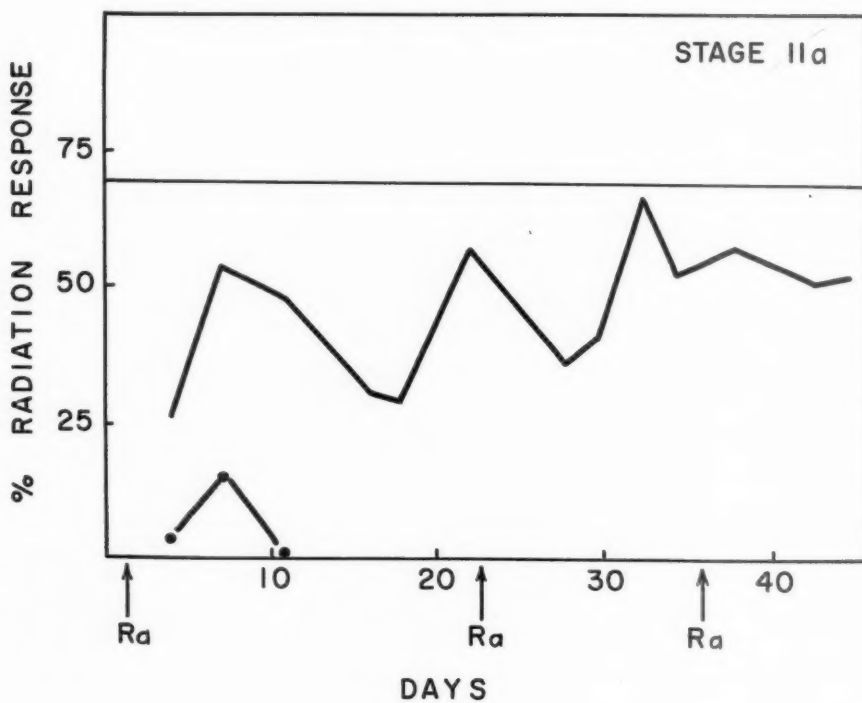


Fig. 4

OLLE KJELLGREN
Gothenburg, Sweden

This is a very interesting, highly complex and far-reaching problem. My investigations can shed light on only some of its aspects. My main interest has been to elucidate the influence of dosage of irradiation from intravaginal radium on radiation cell changes in the vaginal smear. Irradiation for cervical carcinoma according to the Stockholm method means that the dosages of irradiation directed towards the portio and vaginal mucosa are so high that it can be taken for granted that throughout the range of therapeutic variation they will exceed by a wide margin the threshold for production of radiation cell changes. The problem has, nevertheless, been analyzed statistically, taking into account the influence of the dosage (expressed in mgh) of vaginal radium as well as of the radiation dosage (expressed in r-units) measured in the rectal mucosa on the radiation cell changes in the vaginal smear.

Radium dosage

The relationship, if any, between the vaginal radium dosage at the first radium treatment (Ra I) and the cytological response was analyzed. The initial radium treatment was used as the basis for the comparison because its effect was considered easier to evaluate than that of subsequent radium treatment where summation might constitute a source of error. The vaginal rather than the intra-uterine radium dosage was chosen, since the radiation reaction in the vaginal epithelium presumably would be more dependent on the former. The initial intra-uterine radium dosage averaged 1958 mgh in the patients receiving intra-uterine radium at Ra I and in patients receiving vaginal radium at Ra I; the mean dose was 1722 mgh. Thirty-five patients received a vaginal Ra I application dose of up to 1499 mgh, 118 patients received 1,500 - 1,699 mgh, 45 patients 1,700 - 1,999 mgh and 89 patients 2,000 mgh or more. In other words, 153 patients (53.3 ± 2.9%) received 1,699 mgh or less and 134 patients (46.6 ± 2.9%) 1,700 mgh or more. With this in view, 1,700 mgh was used as the dividing value in testing the relationship between the radiation reaction and the vaginal radium dosage.

In the various categories of vaginal Ra I dose, the following poor response frequencies were obtained: ≤ 1,499 mgh, 53.3 ± 9.1%; 1,500 - 1,699 mgh, 56.1 ± 5.6%; 1,700 - 1,999 mgh, 53.3 ± 7.6%; ≥ 2,000 mgh, 56.1 ± 5.5%. None of these percentages differs significantly from any of the others: A poor cytological response was present in 55.6 ± 4.1% of the patients receiving less than 1,700 mgh of radium vaginally at the first radium application and in 55.2 ± 4.4% of those receiving 1,700 mgh or more. The difference is not significant ($\chi^2 = 0.0034$, $df = 1$, $0.90 > p > 0.80$).

However, the radium dosage was adjusted in accordance with the age of the patient. Hence, a similar analysis may be made after introduction of a suitable age limit, here 45 years seemed convenient. It then appeared that 38.8 ± 4.5% of the patients aged 44 years or under were given an initial vaginal radium dosage of less than 1,700 mgh, while the corresponding figure for those aged 45 or over was 64.7 ± 3.9%. The difference between these frequencies is highly significant ($\chi^2 = 17.810$, $df = 1$, $p < 0.001$). The same analysis for an age limit of 50 years reveals that 42.9 ± 3.9% of the patients aged 49 or under and 69.4 ± 4.4% of those aged 50 or over were given less than 1,700 mgh of radium vaginally at first radium application. This difference too is highly significant ($\chi^2 = 18.369$, $df = 1$, $p < 0.001$). The corresponding figures for an age limit of 55 years are 45.6 ± 3.7% and 70.1 ± 4.9% respectively. Also, in this case the difference is highly significant ($\chi^2 = 14.216$, $df = 1$, $p < 0.001$).

As will be discussed more fully under the topic The Clinical Factors Associated with Good Cytologic Response to Irradiation in this Symposium, my data shows that the incidence of poor cytological responses is significantly higher among young than among old patients, whether the age limit is drawn at 45, 50 or 55 years. Hence, as it would be meaningless to do otherwise, I shall present some data regarding the combined influence of dosage of radium irradiation and age on radiation cell changes.

Age and radium dosage combined

With 45 years as the age limit, the poor response frequency was 66.2 ± 5.6% among young patients given a high radium dose, and 46.5 ± 5.0% among old patients receiving a low radium dose. The difference between these figures is nearly significant ($\chi^2 = 6.497$, $df = 1$, $0.02 > p > 0.01$). The corresponding analysis for an age limit of 50 years yielded results. The poor response frequency was 59.8 ± 5.1% in the young patients receiving high radium doses and 44.0 ± 5.7% in the group of old patients with low dosages. The difference between these frequencies is nearly significant ($\chi^2 = 4.129$, $df = 1$, $0.05 > p > 0.02$). The respective frequencies for an age limit of 55 years were 58.6 ± 4.9% and 42.6 ± 6.3%, the difference being nearly significant ($\chi^2 = 3.857$, $df = 1$, $0.05 > p > 0.02$).

With the adopted age classification, a high poor response frequency tended to accompany low age, even when the radium dose was high, but the differences are not fully significant. Nevertheless, in the age group ≤ 44 years, with a radium dose ≤ 1,700 mgh, the poor response frequency was 66.2 ± 5.6% and in the age group ≥ 55 years with a radium dose ≤ 1,699 mgh, it was 42.6 ± 6.3%. Here, the difference is significant ($\chi^2 = 7.377$, $df = 1$, $0.01 > p > 0.001$) (Table 1).

When the lowest age group with the lower radium dose (≤ 44 years and ≤ 1,699 mgh) was compared with the highest age group with the lower radium dose (≥ 55 years and ≤ 1,699 mgh), the poor responses proved highly significant preponderance among the younger patients, the frequencies being 75.6 ± 6.4% and 42.6 ± 6.3% respectively ($\chi^2 = 11.434$, $df = 1$, $p < 0.001$). There is a nearly significant difference between these age groups with the higher radium dose ($\chi^2 = 4.518$, $df = 1$, $0.05 > p > 0.02$).

Within the lowest and highest age groups, high and low radium dosages were not accompanied by significantly different frequencies of poor responses (≤ 44 years: $\chi^2 = 1.144$, $df = 1$, $0.30 > p > 0.20$; ≥ 55 years: $\chi^2 = 0.00074$, $df = 1$, $0.99 > p > 0.98$).

The poor response frequency tended to be higher in young patients even if the vaginal radium treatment dose was high. The differences in poor response frequency between young and old patients were nearly significant for age limits of 45, 50 and 55 years. The lowest age group exhibited a significantly higher poor response frequency after the higher radium dosage than the highest age group after the lowest radium dosage. Whereas the effect of the age factor upon the cytological response proved significant (see below), the magnitude of therapeutic vaginal radium treatment dosages exerted no significant influence on the level of the radiation reaction.

r-Dosage measured in the rectum

For the last three years we have routinely measured the dosage of irradiation in the rectal mucosa in all cases of cervical carcinoma with a calibrated dose-rate meter. Although we have no definite results as yet, a preliminary analysis shows that the dosage of irradiation in the rectal mucosa, which is approximately equivalent to that in the vaginal mucosa, lacks any apparent association with the radiation cell reaction in the vaginal smear. Similar analyses have been made regarding the association between dosage of irradiation in the bladder and the radiation cell reaction (to be published shortly).

	≤ 44 years ≥ 1700 mgh	≥ 55 years ≤ 1699 mgh	Total	χ^2	p	Significance
Poor response	47	26	73	7.377	0.01	Significant
Good response	24	35	59		$> p >$ 0.001	
Total	71	61	132			

Table 1. : Distribution of poor and good responses in the age group ≤ 44 years with ≥ 1700 mgh vaginal Ra I dose and in the age group ≥ 55 years with ≤ 1699 mgh vaginal Ra I dose.

Bibliography

1. Kjellgren, O.: Acta Radiol. Suppl. 168, 1958.

DISCUSSION

JORGE CAMPOS R. de C., Lima, Peru:

Ruth Graham emphasizes the importance of a factor to evaluate the RR, which is the radiation dosage received at the time when the RR is determined. This is for patients under x-ray treatment. For patients with radium treatment it is essential to know the time elapsed since the treatment, because RR is not permanent.

RR increases with higher radiation dosage; this observation is coincident with the one of Kjellgren in the topic entitled "The Clinical Factors Associated with Good Cytologic Response to Irradiation" of this Symposium.

From a practical point of view, it is not possible to evaluate the RR every day in a large series of patients. For this reason the suggestion to do it only during the ninth to sixteenth day or only on the fourteenth day is more practical and seems to fit better into the routine work.

J. EDWARD HALL, Brooklyn, New York, U. S. A. :

The biological response of a malignant tumor is related not only to the amount of radiation delivered to the tumor but also to the time during which a quantity of radiation is given.

Therefore, it would seem that the radiation response as determined in the normal cells might follow the same pattern.

At our institution, there was a distinct change in the method of administering x-ray therapy with the advent of a new professor of radiology. In brief, the main change consisted in shortening the interval during which the x-ray therapy was given.

In evaluating the radiation response after 1000r, 2000r and 3000r had been administered to the tumor, the responses were not the same for the two methods, although the number of roentgens at the tumor were the same.

Thus, we believe that although the amount of radiation is the most important aspect, the time factor during which it is given is also important when x-ray therapy or radium is the first modality employed.

PIERRE HAOUR, Lyon, France:

Everyone who has ever tried the quantitative evaluation of irradiated smears will be impressed by the results of Ruth Graham and Kjellgren.

Following patients regularly after irradiation by repeated cell counts is not a simple task for a routine laboratory. My own experience is limited to some selected cases which were followed in order to study the changes in the Feulgen reaction after irradiation and also some preliminary observations which were made on the irradiated cells of urine sediment.

Ruth Graham shows that the degree of radiation cell changes evidently depends upon the dosage of x-rays. Kjellgren, on the other hand, shows that this is not evident for radium therapy; however, the new variable of patient age is introduced, which is interesting (but does not simplify the problem!).

The authors in their answers do not give any information concerning the duration of the cell response in relation to the degree of irradiation. In our experience we noticed that the effect lasts longer if x-rays are associated with the radium, but this seems to depend mostly on individual factors. In the urinary sediment the response seems to be different than in the vaginal smears. (I am anxious to learn of Kjellgren's experience.) In urine, megalocytosis always appears qualitatively more marked; the response could be observed as early as the time of the first radium application. One of our cases was found to have a 48% response on the third day of radium application. On the other hand, one case still had a 60% radiation response 41 days after radium treatment.

GRACE HERMAN, New York, New York, U. S. A. :

There is no question that it is essential to relate the percentage of radiation response to the amount of external irradiation delivered to the cervix, and similarly to relate the percentage of radiation response after radium insertion to the time interval which had elapsed from the time of insertion. We too have found a series of readings essential for a utilization of the RR phenomenon, a single post-radiation smear at an appropriate dosage or time interval giving the necessary information on only rare occasions.

A report on the correlation in our cases of RR percentage with the ultimate prognosis following a complete course of radiotherapy is not yet available because of the relatively short follow-up intervals. It is worth noting, however, that in a few cases we have seen progression of disease on radiotherapy, or recurrence within a short-time interval, following completion of radiotherapy in spite of a good radiation response. This involves, of course, only a few individual cases and final evaluation must wait an adequate number of cases followed for a sufficient period of time.

OLAF T. MESSELT, Oslo, Norway:

The standard method of treating carcinoma of the cervix at the Norwegian Radium Hospital is by primary radium application in the uterine cavity and against the cervix in one continuous dose for 120 hours, totaling 7600 mgh. This treatment is then either immediately followed by x-ray treatment, 3000 r on each of four fields, giving a calculated tumor dose of 2000 r, or the patient is dismissed from the hospital for six weeks and then readmitted to decide whether further treatment shall be surgery or x-ray.

Due to the method of treatment mentioned above, my experience, as to radiation changes following radium treatment only, is restricted to changes seen in smears taken immediately after completion of the radium application and those in smears taken six weeks later. In smears taken immediately after radium treatment the cells usually show very little response. This confirms the statement of Ruth Graham that it takes time for radiation cellular changes to develop after primary radium treatment.

Smears taken six weeks after completion of radium treatment sometimes show good response and sometimes poor response. If, however, x-ray therapy is introduced in the latter cases, some of these poor response cases will turn "good." This shows that one cannot determine prognoses from smears taken at such a long interval after radium treatment.

The large majority of smears, which I see, are smears taken after completion of both radium and x-ray therapy and smears taken later on at each follow-up. In these cases, when it is a good response, the cellular changes may be seen for years afterwards.

In a brief paper later in this volume I have used a borderline of 60% for differentiating between "good" and "poor" response in my investigation. This is in agreement with Figure I in Ruth Graham's paper which indicates a level of 50% for 2000 r at the cervix.

At our hospital during the last year we have treated the patients, after initial radium application, with Betatron (31 mev) and with a tumor dose twice as high as our previous x-ray therapy. We are following these cases with smears, but it is as yet too early to draw any conclusions.

CLOSING REMARKS

RUTH M. GRAHAM:

To Dr. Campos: I would agree that for practical purposes and routine work the smears after radium application should be taken from the ninth to the sixteenth day. However, if the first treatment is x-radiation, the smear should be taken when the tumor dose at the cervix is 3000r, and the day this dose is reached would of course depend on the rate of administration.

To Dr. Herman: Unfortunately, it is true that an occasional good response will have progression of the tumor during radiotherapy or a rapid recurrence of the disease. Having a good cytologic response to radiation by no means guarantees survival, as can easily be seen from all the series quoted in this symposium. It does mean that the patient has a significantly greater chance of five year survival than with a poor response.

To Dr. Hall: We have been able to compare two series of cases in which the radiation was delivered for two different periods of time. At the Massachusetts General Hospital the daily tumor dose administered was about half that given at the present time at the Roswell Park Memorial Institute. The daily tumor doses in most of the cases at the former hospital were from 87-120r per day, while here the daily dose ranges from 185-225r. We have seen no difference in response of the two groups, even though one group received the radiation in half the time. The division between poor and good have remained the same at any specified dosage.

To Dr. Haour: I assume that it is Kjellgren who introduced the question of the age of the patient, since I made no comment on that point. Dr. Haour points out that nothing was said about the duration of response. The marked response to radiation is a temporary phenomenon and does not last long. I am referring to counts over 75%. However, residual radiation changes may last for years. In reviewing some cases recently that had been treated as long as twenty years ago there were cells which were interpreted as having radiation changes (1), though not in great numbers.

To Dr. Messelt: As mentioned above I agree with Messelt that in those patients with a good response to radiation the cellular changes may be seen for years afterward. In the poor response group, on the other hand, it is sometimes difficult to see any radiation effect even a few months later.

Bibliography

1. Graham, J.B., Graham, R.M., Sotto, L. and Bailey, N.: Radiology (in press).

OLLE KJELLGREN:

As Hall points out, the biological response of a malignant tumor is dependent on the radium dose and the time of treatment, i.e., the method used and the fractionation. It is evident that observations concerning the radiation reaction in the vaginal smear from one center with a certain method of radiotherapy cannot be transferred to the material of another center where patients are treated according to a different method with a different relationship between dosage and time.

I am sorry to not have any definite answer to give to Haour's question concerning the duration of the radiation cell changes in the vaginal smear after different amounts of irradiation. I have not performed any systematical analysis concerning this problem, nor have I had any experience with the radiation changes in urinary sediments.

CAN THE CYTOLOGICAL OR HISTOLOGICAL RESPONSE TO IRRADIATION BE INFLUENCED BY MEDICATION OR OTHER FACTORS?

RUTH M. GRAHAM AND JOHN B. GRAHAM

Buffalo, New York, U.S.A.

Though most of the interest in the radiation response has been in the practical application of a prognostic method, it may be that the theoretical implications are of equal, if not more importance. There is wide variation in the response of the benign squamous cells to ionizing radiation. The cells of one patient may respond in a dramatic fashion to a small amount of radiation, while by contrast the cells of another will show little or no response after a great deal of radiation. We do not know why the cells respond in such a fashion or why they fail to respond. It occurred to us that since the response is taking place in the benign cells, it might be possible to influence the cells in such a way that the poor response could be converted to a good response.

Since we are dealing here with the response in benign cells - not tumor cells - it was possible to use total body radiation in mice as a test for compounds which might influence the response to radiation. We postulated that if a compound would increase the mortality rate in mice given total body radiation, the same compound might make the patient with cancer of the cervix more sensitive to radiation. In other words, if more mice died from total radiation after receiving an injection of a compound, it probably indicated that the compound had made the mice more sensitive to the radiation. We used as a test dose 400r of total body radiation. In 220 control female animals, we found that this amount of radiation gave a mortality rate of 30% at 40 days. With this standard for comparison we began to test a series of substances, the majority of them steroid hormones or vitamins. We found that two substances did influence the mortality rate in a significant fashion, testosterone propionate and alpha tocopherol (Vitamin E). Testosterone propionate increased the mortality rate to 68% and alpha tocopherol to 80%. Both of these figures were statistically significant (1).

After we had determined that we could alter the death rate from total body radiation by the addition of these compounds, we tested this hypothesis on five patients who had an initially poor cytologic response to radiation. Testosterone propionate was used in four cases and alpha tocopherol in one (2). We were able to show that the response could be modified and changed to a good response in four of the patients. Two of the four patients who had a changed cytologic response are alive at five years.

After it was found that it was possible to alter the response to radiation, we studied a series of 99 cases at the Radiumhemmet in Stockholm with the kind collaboration of Hans-Ludvig Kottmeier. In this series of cases we determined whether or not the patients had a good initial cytologic response to radiation; if they did, no medication was given. If they did not, either testosterone propionate, 25 mg. intramuscularly, three times a week was given or 100 mg. of alpha tocopherol per os daily. Our purpose in this investigation was to determine whether or not we could change a substantial proportion of the poor responses to good cytologic responses, and if we could change them, was the survival as good as in the spontaneous good response without medication. Table I gives the four year results of this study (3).

In Table I the four year results for 99 consecutive cases of primary cancer of the cervix are given according to their cytologic response. The results in the poor response converted to good response group are almost identical to that of the spontaneous good response. If a patient can be converted from a poor response to a good response, the prognostic significance of the cytologic method is similar to that for the spontaneous good response. This impression is substantiated by cases studied, since this series where the one, two and three year survival is similar for the spontaneous good responses and the poor responses converted to a good response.

TABLE I

Initial Cytology	Supplemental Medication	Final Cytology	No. of Patients	No. Free of Disease at 4-5 Years
Good	No	Good	41	24 (59%)
Poor	Yes	Good	34	22 (65%)
Poor	Yes	Poor	12	4 (33%)
Poor	Yes	Unknown	5	5
Poor	No	Poor	6	4
Unknown	No	Unknown	1	1
			99	60

Cancer-of-the cervix patients divided according to their initial cytologic response. Some received supplemental medication; i. e., testosterone propionate - 25 mg three times a week or alpha tocopherol - 100 mg daily, per os. The final cytology was the radiation response observed during the latter part of radiotherapy.

Figures 1 and 2 show radiation curves for two patients, both originally a poor cytologic response to radiation. One was converted to a good response by the addition of alpha tocopherol; the other case was not. The first case is living and well at four years, the second died of her disease within a year of treatment.

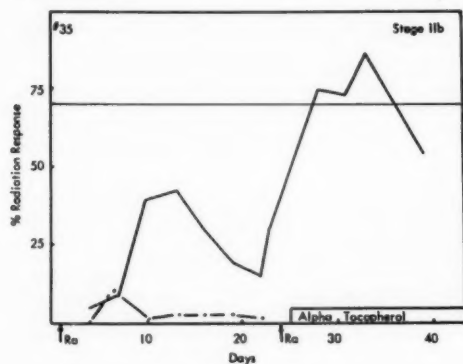


Fig. 1

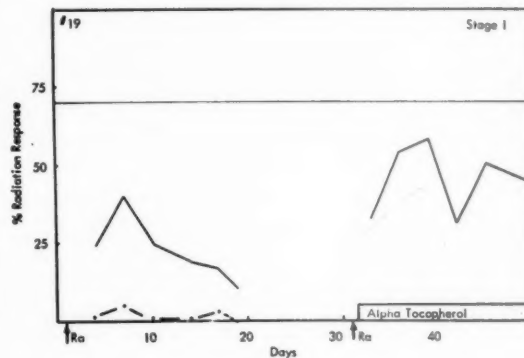


Fig. 2

Unfortunately, not all patients can be converted to a good response. With radium as the primary treatment, about half of the poor responses will be in the good response range after the addition of either testosterone propionate or alpha tocopherol.

Bibliography

1. Graham, J. B. and Graham, R. M.: *Cancer* 3:709, 1950.
2. Graham, J. B. and Graham, R. M.: *Cancer* 6:68, 1953.
3. Graham, J. B., Graham, R. M. and Kottmeier, H. L.: *Acta Union International Contre le Cancer*, (in press).

B. CORNELIS HOPMAN*

Miami, Florida, U.S.A.

According to the Grahams (1), some basal and intermediate cells of the vaginal epithelia in cervical smears indicate sensitization response (SR), an immune response of the host to the cancerous process.

*This work was supported by a research grant from the National Cancer Institute of the National Institutes of Health, United States Public Health Service. Presented at a research seminar of the University of Miami Medical School, Jan. 30, 1959.

These cells have a dense cytoplasm, stain cyanophilic and are finely vacuolated. If a patient has 10% or more of these cells on the slides the prognosis is said to be good (73% cure rate); if 9% or less, the prognosis is poor (18% cure rate). The percentage of these cells rises with the advancing stage of the disease and declines again in far advanced stages because of the loss of resistance when the tumor undermines the patient's defenses. Regional metastases increase SR. Cancer in an early stage has a low SR. SR should therefore be able to give indications for the therapy. Carcinoma in situ with low SR should be treated with surgery. More advanced cancers with higher SR should be treated with radiotherapy which can cure a number of cases with regional metastases. In neglected or late diagnosed cases the patient's defense mechanism and resistance are so undermined that she is no longer capable of producing the minimum response necessary for successful radiation therapy. It follows that her chances for successful surgery are likewise poor. It is in such apparently hopeless cases as these that testosterone and progesterone, which increase SR, can still have some temporary success to lengthen life.

The cytologic diagnosis of radiation response has become a topic of much controversy. Some authors contend that the whole approach to the problem is unsound. They maintain that prognosis depends primarily upon the particular form and extent of the tumor. Osborn (2) in his chapter on radiation response states that the cytologic changes, as described by cytologists, are practically incomprehensible and unnecessarily complicated. It is quite sufficient to relate radiation response to estrogenic activity, in such a sense that a smear of full estrogenic activity is changed to a smear of estrogenic deficiency. If the smear before radiation was lacking in estrogen, as in older women, the changes must obviously be unconvincing. It is possible that the indirect effects of radiation on the ovaries causing estrogenic deficiency are of greater importance than the cytologic changes.

In a recent meeting of the Inter-Society Cytology Council in New York (Nov., 1958), Grace Herman (3), as a result of studies of 200 cancer cases, came to the conclusion that SR is a nonspecific response closely allied to the endocrine status of the patient rather than a specific response to carcinoma. Fennell (4) using a fluorescent antibody technique, found that no specific antibody was detected in given vaginal cells, as related to sensitization response.

The author examined fifty slides a week during the last four years of patients receiving radiation therapy for cervical cancer in all stages, several of them for five years or longer. Six specific features were observed. They were tumor cells, radiation reaction (RR), sensitization (SR), cornification, number of squamous cells and infection (leukocytes). Tumor cells, of course, indicate a poor prognosis, especially of the undifferentiated type of cancer cells. These cells may easily be missed, causing negative diagnoses. They are small cells with only a slight rim of cytoplasm. They often indicate a far advanced cancer. The results show further the significance of RR, but there were many exceptions (cure rate of approximately 70% in the good response group and 40% in the poor response group). The SR is of greater significance especially in the poor response group (cure rate of approximately 70% in the good response group and 20% in the poor response group). An increasing cornification of the cells is a poor prognostic sign. Of great importance are clean slides. Progressive infection marked by increasing amounts of leukocytes with relative decreasing numbers of squamous cells is a poor prognostic sign. The absolute number of leukocytes is not of so much importance as the ratio between increasing numbers of leukocytes and decreasing numbers of squamous cells. In many unfavorable cases we find only a relatively small number of cornified squamous cells buried under a mass of leukocytes. Low SR as represented by a high estrogenic reaction is probably caused by an increased concentration of estrogens in infections tissue rather than specific response to carcinoma (Figs. 1, 2). Brunelli (5) as early as 1935 found that estrogens concentrate in infected tissue. Before the antibiotic era we treated some gonorrhea in children and infections in general with estrogens. Smears of a normal cervix show differences in the number of leukocytes present before and after ovulation, presumably due to a difference in estrogenic level (6). That infection plays a role in the etiology of cancer is shown by the disruption of the normal cell pattern associated with chronic infection including *Trichomonas vaginalis*. The nucleus becomes irregular in size and shape. There is some hyperchromatism and irregu-



Fig. 1

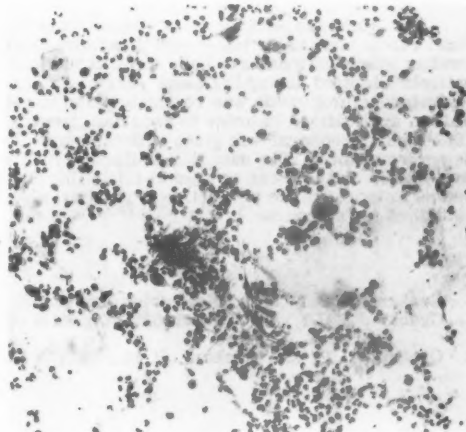


Fig. 2

larly divided chromatin. A halo formation is found around the nucleus. It is correct that the prognosis of cancer is primarily dependent upon the extent of the tumor and the type of its cells, but infection influences the process in an adverse way. This is caused by the increased lymph and blood supply, the greater infiltration with fluid and the loosening of the contact of the malignant cells by the hyaluronidase enzymes of the responsible bacterial and viral agents (7, 8, 9, 10).

The influence of these considerations on medication is evident. Prevention of cervical cancer includes these points: avoiding all irritations, careful treatment of wounds, lacerations and erosions of the cervix whether they be caused by abortion, childbirth or infection, and a careful treatment of chronic cervicitis. When cervical cancer has occurred and been radiated, the treatment does not end with the radiotherapy and awaiting the five year result. If signs of increasing estrogen appear and the basal and intermediate cells become eosinophilic, it is possible that hormone therapy can counteract these changes. Whether this be in the form of progesterone or testosterone or whether extirpation of the ovaries should be performed must be decided by the clinician. Of greatest importance is the treatment and avoidance of infection, which is probably the most important factor contributing to invasion and metastases. Infection may be treated by estrogens temporarily, by antibiotics, sulfa preparations, etc. *Trichomonas vaginalis* should be definitely irradiated. Although in itself it is probably not a cause of cancer, it supplies irritating factors adding to the adverse influence on a malignant process. X-ray therapy, in preparation for local cobalt or radium therapy, is the procedure of choice in most cases, except possibly the very earliest which have a minimum of infection. The local infection is markedly reduced by external x-ray.

CONCLUSION

In cytological examination of cases of irradiated cervical cancer recurrence of cancer cells represent an unfavorable prognosis, especially cells of the undifferentiated type. Increasing cornification and infection (measured as a ratio of the number of leukocytes to the number of squamous cells) is an unfavorable sign in the course of a malignancy. Therapeutic measures should be secured to counteract these unfavorable signs.

Bibliography

1. Graham, J. B. and Graham, R. M.: *Annals of the New York Academy of Sciences* 63:1458, 1956.
2. Osborn, G. R.: *Applied Cytology*. London, 1953, Butterworth and Co.
3. Herman, Grace G.: *Sixth Annual Meeting of the Inter-Society Cytology Council*. Nov., 1958.
4. Fennell, R. H. and Vazquez, J. J.: *Sixth Annual Meeting of the Inter-Society Cytology Council*. Nov., 1958.
5. Brunelli, B.: *Archives Internationales de pharmacodynamie et de therapie* 49:295, 1935.
6. Hopman, B. C.: *Over de betekenis van het vaginale uitstrykpreparaat in verloskunde en gynecologie* thesis, Excelsiors foto offset, s'Gravenhage, 1951.
7. Balasz, E. A. and von Euler, J.: *Cancer Research* 12:362, 1952.
8. Greenstein, J. P.: *Biochemistry of Cancer*. New York, 1954, Academic Press Inc.
9. Kiriluk, L. B., Kremen, A. J. and Glick, D.: *J. Nat. Cancer Inst.* 10:993, 1950.
10. Stoppelman, R. H.: *Acta paediatrica* 39:510, 1950.

OLLE KJELLGREN

Gothenburg, Sweden

Ruth and John Graham's researches suggest that the radiation cell reaction in irradiated cervical carcinoma can be influenced by administration of alpha tocopherol and testosterone propionate. Similar investigations have been made at the Radiation Centre, Gothenburg, in which we have given all patients admitted during the same year a particular adjuvant therapy. Thus, in the years 1954 and 1955 testosterone propionate was routinely given to patients with cervical carcinoma in conjunction with the radium applications in order to ascertain its effect on the radiation cell reaction. Similarly, in 1956 and 1957 alpha tocopherol was given with the same object in view. Currently a combination of both drugs is being prescribed. The data thus collected will be analyzed when all the patients have been observed for five years. All that can be said at this point is that neither alpha tocopherol nor testosterone propionate seems to increase the proportion of good cytological responses in our material. (Full results will be published in due course.)

Bibliography

1. Graham, J. B.: *J. A. Geriatrics Soc.* 1:567, 1953.
2. Graham, J. B.: *The Laboratory Diagnosis of Cancer of the Cervix* (Homburger and Fishman). Basel, 1956, S. Karger.
3. Graham, J. B. and Graham, R. M.: *CA. A Bulletin on Cancer Progress* 5:56, 1955.

DISCUSSION

WARREN R. LANG, Philadelphia, Pennsylvania, U.S.A.:

The outstanding work of the Grahams in studying the prognostic value of the vaginal cytologic smear in determining future response to irradiation (SR) and the response during and after radiotherapy in cervical carcinoma (RR) is well known to the average cytologist. Controversy has revolved around SR rather than RR.

The Grahams' experiments with total body radiation in mice are convincing. Results in humans demonstrating a trend toward increased salvage with testosterone propionate and alpha tocopherol must await further confirmation from more five year "cures" and from the work of other investigators. Kjellgren, in a preliminary report, is unable to confirm the findings of the Grahams. Hopman contends that staging is still a determinant of prognosis, a view in which I am sure there is practically universal concurrence. Inflammatory changes and the persistence of carcinoma cells are indicative, cytologically, of poor response to radiotherapy.

It is probable that "radiation response" like "estrogen response" is not a specific reaction, although both are usually associated with radiation and estrogen respectively. It is interesting and probably significant that some radiomimetic effects are noted with chemotherapeutic agents (1) and that what are called radiation changes mirror in some way the response of the host to the tumor.

Bibliography

1. Schmidt, L. H.: Annals of the New York Academy of Sciences, 68:657, 1958.

NO CLOSING REMARKS

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

SHOULD A LESION BE TREATED WITH SURGERY AFTER IT HAS BEEN SHOWN CYTOLOGICALLY THAT THERE IS NOT ANY, OR ONLY SLIGHT, RADIATION RESPONSE PRESENT?

JOHN B. GRAHAM
Buffalo, New York, U.S.A.

Patients with a poor RR have a poor prognosis when treated radiologically. For the past four years we have been operating on patients who show poor RR early in the course of radiotherapy. In Table I are listed the data on three groups of patients: 1) The Boston Series in 1954 - 56 at four hospitals: Massachusetts General, Free Hospital for Women, Pondville Hospital and the Peter Bent Brigham Hospital. Follow-up on these patients is 2 - 4.5 years. 2) The Buffalo Series in 1957 and 1958 at Roswell Park Memorial Institute. The first of these has a one year follow-up.

TABLE I

CANCER OF THE CERVIX, STAGES I, II, III								
	Primary Surgery		Poor RR Surgery		Poor RR No Surgery		Good RR	
	No.	L & W	No.	L & W	No.	L & W	No.	L & W
Boston (2 - 4.5 year follow-up) 1954 - 56* Stage I, small Stage I - III	37	34	28	22	9	0	114	69
								*excluded are 3 primary surgery, with Marked SR
Buffalo (1 year follow-up) 1957 Study Series Stage I, small Stage I - III	7	7	10	6	6	2	28	24
Radiation Series Stage I, small Stage I - III					7 22	5 10	4 22	4 16
Buffalo 1958 Study Series Stage I, small Stage I - III	6		18		2		28	
Radiation Series Stage I, small Stage I - III					4 15		22	

Patients with a Stage I lesion no larger than 2.5 cm. and poor SR were operated upon primarily. All other patients had a trial of radiation. In Boston the radiotherapy was two applications of radium three weeks apart followed by x-ray (Modified Stockholm technique) (2). In Buffalo the radiotherapy was full pelvis x-ray, 1000 r at the cervix per week followed by a small dose of radium. Half of the Buffalo patients were treated by radiotherapy alone as controls. In Table I can be seen the distribution of cases in the three series and the symptom free rate in the first two series.

Kind of Surgery:

We have restricted our surgery to a radical hysterectomy and regional lymphadenectomy - the operation of Meigs (1). In a few patients the lymph node dissection was omitted. Exenteration was not employed.

The RR can be determined 14 days after the first application of radium or at 1000 r in the region of the cervix with full pelvis x-ray. If the RR is found to be poor, further radium is omitted or further x-ray stopped. The total dose of x-ray is about 1500 r in the Buffalo Series. Surgery should be done as soon as possible after recognition of poor RR. In our hands, the average time is one week. Two patients who were allowed to go two months after stopping x-ray were both found inoperable.

The smallest amount of radiation that will determine the response is also desirable. In Table II is summarized the Boston experience. Nineteen were operated upon after less than one-half the full dose of radiation and 16 are living and well. Six had more than half the full course prior to operation and four are living and well. One of the patients who died received a full course of radiation - belatedly we decided that the RR was poor and a radical hysterectomy was done two months after radiotherapy was completed. She developed a recto-vesico-vaginal fistula and died of hemorrhage four months post operative. Three patients had persistent disease after radiotherapy and were operated upon within four months - two are living and well.

TABLE II

RADICAL HYSTERECTOMY AFTER SOME RADIOTHERAPY BOSTON SERIES						
	Less than Half Full Radiotherapy		More than Half Full Radiotherapy		Full Radiotherapy Surgery for Persistent Disease within 4 Months	
	No.	L & W	No.	L & W	No.	L & W
Stage I	7	5	2	1	2	1
Stage IIa	8	8	1	1	1	1
Stage IIb	4	3	2	1		
Stage III			1	1		
	19	16	6	4	3	2

We believe that patients with poor RR should have a radical hysterectomy and regional lymphadenectomy if possible. Surgery should be done within three weeks of a single application of radium (where two applications are the definitive treatment) or within ten days of stopping roentgen therapy, where a dose of 1000 - 1500 r has been delivered in the region of the cervix in 7 - 10 days.

Bibliography

1. Meigs, J.V.: Surgical Treatment of Cancer of the Cervix. New York, 1954, Grune and Stratton.
2. Schulz, M. and Graham, J.B.: Progress in Gynecology, 3:565. New York, 1954, Grune and Stratton.

DISCUSSION

S. B. GUSBERG, New York, New York, U.S.A:

If radiation response is to have validity for transfer of patients to a surgical mode of treatment, the radiation should be a test rather than a full treatment and the time lapse should be short. In these conclusions our studies with tumor response concur with Graham's work with normal cell response.

We have found 3000 r delivered in five days by trans-vaginal cone to be a more usable tumor cell test than an initial radium dose or external x-ray therapy; the post-radiation tumor sample is then taken 12 days after the onset of trans-vaginal cone. If the response is poor or mixed in Stage I or Stage IIa, the patient is subjected to radical hysterectomy within three weeks. We do not believe this amount of radiation or time interval interferes with proper surgical treatment and it may even have a beneficial preoperative effect on the tumor surface.

It is logical to assume that testing will help us define the proper role of radiation and surgery in the treatment of cervical cancer. But the problems of radiosensitivity and radiocurability are so complex that it is difficult to forecast the precise improvement in cure rate to be anticipated from their solution.

J. EDWARD HALL, Brooklyn, New York, U.S.A.:

The question of when to operate on patients with carcinoma of the cervix who show a poor RR is most important. The Grahams have presented evidence that the RR is significant enough to warrant interruption of radiotherapy and institute surgery. At our clinic we are beginning to find a close correlation between the RR and the final outcome. However, at present, we hesitate to rely on the RR to the extent of stopping radiotherapy. We have had the experience of patients with poor RR determinations and even poor clinical response during and immediately following radiotherapy only to show marked resolutions a few weeks later.

All of our patients with Stage I involvement are treated surgically. As yet we are hesitant to stop radiotherapy in the more advanced lesions because of a poor RR. However, a poor RR does influence us to operate sooner now, if at the completion of radiotherapy there is also an unsatisfactory clinical response.

Time and more cases may prove Graham correct.

OLLE KJELLGREN, Gothenburg, Sweden:

From the clinical point of view this is a most important question as far as radiation cytology is concerned. When commencing the study of the radiation reaction in the vaginal smear at the Radiation Centre in Gothenburg in 1950, this problem was our main interest. Our investigation concerning the prognostic significance of the radiation reaction in the vaginal smears from patients treated for cancer of the uterine cervix according to a modified Stockholm method, showed that the five-year apparent recovery rate is $36.5 \pm 4.3\%$ in the poor response group and $73.9 \pm 4.1\%$ in the good response group, the differences not being as impressive as those found by the Grahams. According to our experience in prognosis for the good response group is not really good even if it is definitely better than in the poor response group. The frequency of local recurrences in the poor response group was $29.7 \pm 4.8\%$ and in the good response group $7.1 \pm 2.4\%$. The investigation has shown that even in the good response group, one may expect local recurrences though they are few and also that in about 26% of the patients with a good cytological response a five-year healing rate will not be obtained. With these facts in mind we have not considered radical surgery a final solution of the problem in the poor response group only as part of the good response cases will succumb, and about 36% of the patients showing a poor cytological response will obtain five-year healing without surgery. Instead, we have been trying to change the patient's response to radiation by various means. At present a series of patients are prophylactically oophorectomized before radiation therapy with the view of studying if this procedure might cause an improvement of the cytological response. There are certain reasons for this (1).

Bibliography

1. Kjellgren, O.: Acta Radiol. Suppl. 168, 1958.

RADICAL SURGERY IN CASES OF RECURRENT CERVICAL CARCINOMA AFTER IRRADIATION

JUAN CARLOS AHUMADA

Buenos Aires, Argentina

The following results were observed in 354 carcinomas of the uterine cervix, which had been treated between 1942 and 1949 with irradiation therapy (radium therapy according to the Regaud technique and preventive x-ray therapy). Of 224 patients, 119 cases of recurrent disease were seen; (48.7%) 84 were localized, 7 metastatic and 28 not specified.

The local recurrences were classified as follows:

- I. Recurrence "in situ": with clinical localization in the cervix, or with moderate spread to vaginal cavity and lateral parametria (Stages I and II): 37 cases (44.0%).
- II. Local anterior recurrence, with spread to anterior parametrium (precervical space, anterior vaginal fornix and eventually to bladder) 6 cases (7.0%).
- III. Local parametrial recurrence involving lateral and/or posterior lateral parametria (Stages II and III): 37 cases (44.0%).
- IV. Local posterior recurrence eventually involving the rectum: 4 cases (4.7%).

When radical surgery was deemed possible (Wertheim operation with ganglionic pelvic evisceration; Brunschwig operation) the following therapeutic pattern was followed:

1. Wertheim operation is the treatment of choice in recurrent cases of carcinoma "in situ" (cervix) in Stages I and II, if and when the cases are technically operable without being unduly taxing.
2. In recurrent local anterior cases, pelvic anterior evisceration has to be carried out.
3. In recurrent local posterior cases, pelvic posterior evisceration has to be carried out.
4. In recurrent local cases involving the cervix, the vaginal cavity, the anterior, lateral and posterior parametria, total pelvic evisceration has to be carried out.

In all cases the total invasion of the parametria (frozen pelvis) is a contra indication for the operation.

According to our experience, primary mortality in pelvic evisceration (during the first month) was about 24%; survival after two years was 30.7%. The survival rate after five years was 15.3%.

JOHN B. GRAHAM

Buffalo, New York, U.S.A.

Radical hysterectomy for recurrence after full, adequate radiotherapy is of limited value. In 100 patients with recurrence we found that only seven were suitable for radical hysterectomy and only one of those lived five years (1). In the past two years at Roswell Park Memorial Institute (where we see 130 primary cancers of the cervix a year and an additional 50 patients with cancer of the cervix previously

treated) we have performed radical hysterectomy seven times for recurrent cancer. A major factor is the low frequency confined to the cervix or immediate vicinity. Radical hysterectomy of the Meigs' type in patients who have had radiation in full dosage is hazardous as exemplified by two double fistulas in the seven patients (our fistula rate in patients with no or only partial radiation is 5%).

The development of a positive smear in a patient previously treated with radiation almost always is followed by clinical recurrence. However, the tumor is usually active deep in the pelvis or elsewhere and is not amenable to radical surgery.

Bibliography

1. Graham, J. B. and Hendrick, G.: Surg. Gyn. & Obst., 105:482, 1957.

JOE VINCENT MEIGS

Boston, Massachusetts, U.S.A.

It is inevitable that there will be a certain percentage of local recurrences of cancer of the cervix after carefully planned and given radiation therapy. The percentage will be, as a rule, between five and fifteen per cent. It is also inevitable that about the same recurrence rate will appear after primary radical surgical treatment of this lesion, usually not over 10-15%. Radiation or surgery can cure the recurrence after either type of treatment. Perhaps with greater care in the selection of patients for the proper type of primary treatment the recurrence rate may drop.

In the case of local recurrences after radiation therapy, surgery can be carried out in most instances. The type of surgery will vary from simple total hysterectomy to total exenteration. Total hysterectomy will never be the proper treatment, but in some patients the surgeon may find it impossible to carry out the operation he has selected and will have to settle for removal of the uterus and cervix only. There is no doubt that full radium and x-ray therapy may cause such extensive fibrosis and diminution in blood supply that any surgical undertaking may be difficult, as well as dangerous. On the other hand, the operation may be nearly bloodless and appear to be not too difficult; in such instances a break-down in the ureter, bladder, or rectum may follow because of the lack of a good blood supply. If the recurrence is local and just confined to the area of the vagina, the surgical attack can be either vaginal (such as the Schauta operation, or vaginal removal of the recurrence without regard to fistula formation -- the fistula to be repaired later) or abdominal, with an attempt to save the bladder and rectum with the realization of possible fistula formation to follow, which may be treated later. When it becomes clear during the operation that the separation of the cervix and lower uterus from the vagina is impossible due to disease, the anterior exenteration operation can be carried out with the individual choice of urinary diversion. However, if it is obvious that the rectum and areas about it are involved in extensive disease, and if the aortic nodes are negative, then total pelvic exenteration should be carried out with urinary and bowel diversion.

Inasmuch as many postradiation recurrences are accompanied by severe pelvic pain, the operation should be so selected that the area or areas responsible for the pain will be removed. The operation following full radiation therapy may be very difficult, and upon occasions the radical attack will have to be abandoned. The greatest success in the treatment of recurrent cancer following radiation will be in those patients whose recurrence is local, without spread to the pelvic walls. Lateral spread is obviously more serious and its removal much more hazardous and questionable than the recurrence that affect the bladder, rectum, or both. The presence of obvious nodes near and on the lateral pelvic wall make good results very doubtful.

Recurrence should be attacked by the surgeon with the hope that a Wertheim type of operation with bilateral dissection of the pelvic lymph nodes will suffice, but he must be ready to carry out any pelvic operation necessary to eliminate the disease. If it is impossible on exploration to feel that all the cancer can be removed, it is best to abandon the radical surgical attack. The only reason for a palliative operation in this group of patients is one that is designated to eliminate pain. We know that radium will cure a certain percentage of recurrences after full therapy; this fact must be given consideration before the surgeon maps out his surgical procedure.

ARNALDO de MORAES AND HILDEGARD STOLTZ *

Rio de Janeiro, Brazil

Until the end of 1957, 700 patients were treated for carcinoma of the uterine cervix in the Instituto de Ginecologia da Universidade do Brasil, Rio de Janeiro. Of the patients who were submitted to surgery after intracavitary radium therapy, with or without transpelvic x-ray therapy, there were 30 in whom a detailed study of the problem is available. Besides these 30 patients of the public university ward (indigent patients of low socio-economic level), we also have available two private patients. In 16 of the ward patients and in the two private patients the confirmation of the presence of cancer, after complete radical radium therapy, was made before the indication for additional surgery.

*From the Instituto de Ginecologia da Universidade do Brasil, Rio de Janeiro, D. F., Brasil.

The diagnosis was confirmed by biopsy. Yet the experience has shown that cervical biopsy after radium therapy gives no information as to the extension or spread of cancer. The presence of cancer only in the cervix is of much better prognosis in our cases than when there existed infiltration of the myometrium with carcinomatous tissue up to the fundus uteri. One patient in whom the study of the surgical specimen revealed carcinoma in the posterior parametrium and rectovaginal septum, had a recto-vaginal fistula but has been free from cancer for more than 18 months.

Because of persistent or recurrent cancer not all patients underwent surgery; We wanted to study in some of them the efficiency of our technique of radium therapy. Yet even these studies are problematical. Even when the biopsy shows that there still exists cancerous tissue within two months after radical radium therapy, the prognostic interpretation as to radiocurability may be doubtful. (Simone Laborde stated that at least three months must pass before the presence of cancerous tissue in the biopsy after irradiation signifies radioresistance or radioincurability.)

Thus we conclude:

- 1) The correct interpretation of histologic aspects after radium therapy of cervical cancer may offer considerable difficulties.
- 2) A negative vaginal smear after radium therapy does not always exclude the existence of growing cancerous tissue in the parametrium or in the deeper layers of the myometrium.
- 3) Surgery is absolutely indicated after radical radium therapy in patients in whom after three or more months carcinoma still exists in the fundus of the vagina or in the cervix uteri, even if the interpretation is equivocal, and even in cases where there may exist doubts as to whether the cancer is actively growing or whether there are inactive residual nests strangulated in connective tissue.
- 4) The therapeutic results in about 10% of our ward and private patients justify the indication and compensate for the effort and expense of the laborious additional surgery (Wertheim-Meigs or Brunschwig operation) after failure of radium therapy.

DISCUSSION

OLLE KJELLGREN, Gothenburg, Sweden:

Recurrent carcinoma of the uterine cervix after irradiation with adequate doses of intracavitary radium and x-rays to the pelvic walls presents many difficult problems from the therapeutical point of view. The frequency of local recurrences, being about 15% among all primarily healed cases, is almost four times higher among patients showing a poor cytological response than among those with a good cytological response. However, the recurrence of irradiated cervical cancer is not always restricted to the cervix but might occur in the cervix, the parametria and/or the lymph nodes on the pelvic wall simultaneously. The methods of treatment used at the Radiation Centre in Gothenburg are: radical hysterectomy with lymph node dissection, anterior or posterior exenteration; total exenteration is usually not performed. A patient with a recurrence localized to the cervix has a fair chance of remaining cured after radical surgical treatment. With coexisting spread to the lymph nodes on the pelvic wall after previously completed radiation therapy the prognosis is hopeless. I agree with Meigs in believing that a recurrence growing forward to the bladder or backward to the rectum is more apt to heal after radical surgery than a recurrence with lateral spread. As the chances of curing a recurrence after completed radiation therapy usually are poor, owing mainly to the fact that the growth is far advanced before clinical diagnosis, one has been looking for means to diagnose the recurrence in a subclinical stage. At this point the cytological examination of the vaginal smear has been of great value, and in a number of cases it has been possible to prove a local recurrence cytologically long before it has been clinically manifest.

Bibliography

1. Kjellgren, O.: Acta Radiol. Suppl. 168, 1958.

NO CLOSING REMARKS

THE CLINICAL FACTORS ASSOCIATED WITH GOOD CYTOLOGIC RESPONSE TO IRRADIATION

JOHN B. GRAHAM
Buffalo, New York, U.S.A.

Cancer of the cervix with vaginal involvement is favorable, if the lesion is flat (i. e., not deeply ulcerated or fungating), the upper vagina shrunk and conical with obliteration of the fornices and if there is no palpable invasion of the paracervical or paravaginal tissue. This usually is found in older women.

Full pelvic radiotherapy as employed at Roswell Park Memorial Institute results in diarrhea in most patients. An absence of diarrhea or other gastro-intestinal symptoms is associated with a poor RR.

Infection in the pelvis (salpingitis) or elsewhere, (e. g., pneumonia) is associated with a poor RR. Bleeding from the tumor is associated with poor RR. Of eighteen patients who had to be hospitalized for bleeding during radiotherapy, thirteen had poor RR.

OLLE KJELLGREN
Gothenburg, Sweden

In a series of 287 patients with cervical carcinoma, the cytological response to irradiation in accordance with a modified Stockholm method has been correlated with the following factors: international (clinical) tumor stage, duration of history, number of pregnancies, gross tumor morphology, age, and hormonal status as judged by different means.

International tumor stages showed no significant association with the poor or good cytological response observed in the vaginal smear obtained either after the initial radium application or after completed radium treatment.

Duration of history. In classes of 0-1, 2-3, 4-6, 7-11 and 12-24 months the distributions of poor and good cytological responses showed no significant differences.

Number of pregnancies. In groups of patients with 0, 1-3 and 4-10 pregnancies the distributions of poor and good cytological responses exhibited no significant differences.

Gross tumor type. In order to establish if the gross tumor type might have any influence on the cytological response, the patients were classified into four groups with respect to the type of gross tumor. Tumor Type I consisted of neoplasms presenting a superficial ulceration or an endocervical growth, that is, cases where no true tumor could be observed bulging into the vagina from the portio. In these cases the vaginal radium was applied directly to the portio and the vaginal fornices. Tumor Type II was comprised of discoid or crateriform tumors. Tumor Type III contained so-called cauliflower tumors, usually voluminous, which made it necessary to place the radium applicator some distance away from the vaginal fornices. Tumor Type IV included all forms of neoplasms not classifiable under any of the preceding types. As the good and poor response frequencies were not significantly different within any of the tumor type groups, it could not be demonstrated that the cytological response was influenced by the gross tumor type.

Age was correlated with the cytological response expressed in 10% increments of radiation cell changes in the vaginal smear obtained about two weeks after the first (i. e., immediately before the second) radium application. It was found that the mean age increased significantly with rising radiation reaction

levels (Fig. 1). The regression line had the equation: $Y = 42.49 + 0.113x$ ($S_b = 0.024$, $df = 8$, $t = 4.7$, $p < 0.001$).

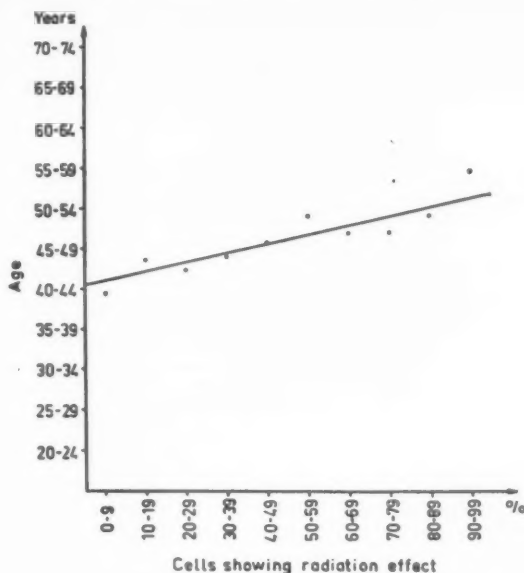


Fig. 1. Mean ages at different response levels.

When the patients were divided into two age groups, there was a highly significant preponderance of poor cytological responses among the younger patients whether the dividing line was drawn at 45 or 50 years, and a significant one when the age limit was 55 years. In the group aged ≤ 44 and the group ≥ 55 years, the poor response frequency was $65.3 \pm 4.3\%$ and $39.4 \pm 5.0\%$ respectively, the difference being highly significant ($\chi^2 = 14.3$, $df = 1$, $p < 0.001$).

Evidently, therefore, the age factor influences the cytological response in the sense that the incidence of poor responses is significantly higher among young than among old patients.

How cessation of menstrual function affected the cytological response was studied by dividing the 287 patients into premenopausal and postmenopausal groups, with poor response frequencies of $58.1 \pm 3.7\%$ and $40.7 \pm 4.7\%$ respectively, their difference being significant ($\chi^2 = 8.1$, $df = 1$, $0.01 > p > 0.001$).

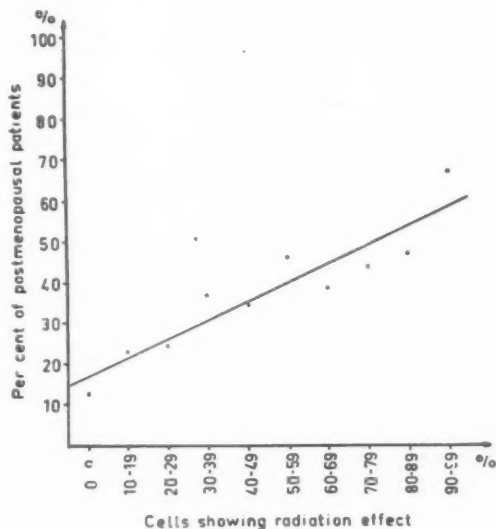


Fig. 2. Postmenopausal patients at different response levels.

Analysis of the frequencies of postmenopausal patients at consecutive 10% increments of radiation cell changes after the first radium application in the smear obtained immediately before the second radium application showed that the frequency of postmenopausal patients became significantly higher at rising radiation reaction levels (Fig. 2). The regression line had the equation: $Y = 14.40 + 0.464 x$ ($S_b = 0.064$, $df = 8$, $t = 7.2$, $p < 0.001$).

Hormonal effect, expressed in arbitrary hormonal levels A (lowest estrogen influence) to E (highest hormonal influence), on the cytological response was studied by comparing a group of patients having hormonal levels A-C with another group having hormonal levels D-E, the corresponding poor response frequencies being $38.4 \pm 4.9\%$ and $58.7 \pm 3.6\%$. The difference is significant ($\chi^2 = 10.4$, $df = 1$, $0.01 > p > 0.001$).

With rising radiation reaction levels in the smear taken about two weeks after the first radium application, the proportion of patients with low hormonal activity evinced by hormonal levels A-C was found to increase (Fig. 3). The regression line has the equation: $Y = 11.70 + 0.502 x$ ($S_b = 0.076$, $df = 8$, $t = 6.6$, $p < 0.001$).

Both the premenopausal and the postmenopausal patients with hormonal levels A-C tended to have lower poor response frequencies than the corresponding groups with hormonal levels D-E. But the difference is highly significant between the $60.1 \pm 4.1\%$ of premenopausal patients with hormonal levels D-E who had a poor response and the $35.1 \pm 5.4\%$ of the postmenopausal patients with hormonal levels A-C who also had a poor response ($\chi^2 = 12.597$, $df = 1$, $p < 0.001$).

Examination of the poor response frequency at high and low hormonal levels in different age classes revealed that the poor response frequency at hormonal levels D-E showed a highly significant excess among young patients, whether the age limit was drawn at 40, 45, 50 or 55 years. The poor response frequency showed no such preponderance among young patients with hormonal levels A-C for any of the above age limits.

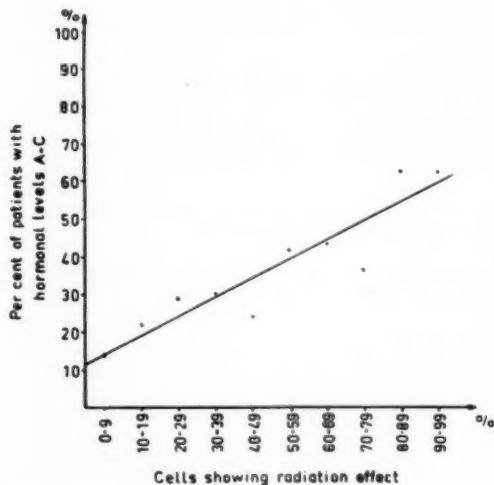


Fig. 3. Patients with hormonal levels A-C at different response levels.

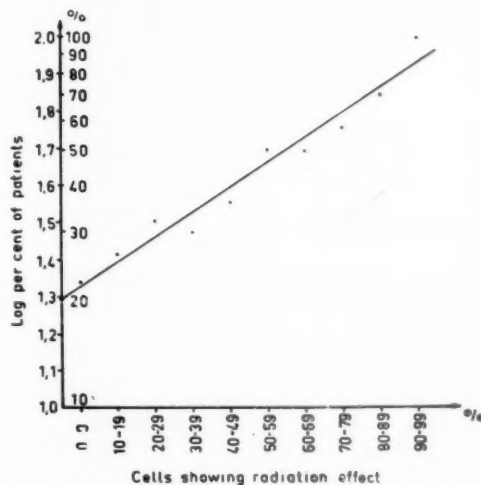


Fig. 4. Patients ≥ 45 years with hormonal levels A-C in relation to patients ≤ 44 years with hormonal levels D-E at different response levels.

When both age and hormonal level were taken into account and the frequencies of patients ≥ 45 years old with hormonal levels A-C and patients ≤ 44 years old with hormonal levels D-E were analyzed at rising response levels, it was found that the proportion of old patients with low hormonal levels increased significantly (Fig. 4). The regression line has the equation: $\log Y = 1.2965 + 0.00666 x$ ($S_b = 0.000489$, $df = 8$, $t = 13.6$, $p < 0.001$) or $Y = 19.79 \cdot e^{0.015x}$.

It has thus been found that the radiation reaction in the vaginal smear lacked any obvious association with any of the following: gross tumor type, duration of history, number of pregnancies, and clinical tumor stage. The degree of radiation reaction was significantly influenced by the patient's age as well as by her hormonal condition, whether it was assessed from her menopausal state of in accordance with various methods for estimating estrogen activity in the vaginal smear. A good response was correlated to rising age, and the good response frequency was higher among postmenopausal patients than among premenopausal patients. Similarly, it was higher among patients with low hormonal activity in the vaginal smear than it was among those with high activity. The proportion of postmenopausal patients, of patients with low hormonal levels and of elderly patients with low hormonal levels all increased with rising radiation reaction levels.

Bibliography

1. Kjellgren, O.: Acta Radiol. Suppl. 168, 1958.

JAMES A. MERRILL AND DAVID A. WOOD *

San Francisco, California, U.S.A.

It has been suggested that the degree of radiation response of normal vaginal epithelial cells is an index of host response to tumor and treatment and not directly an indication of tumor response. Thus, it may be anticipated that clinical factors will influence the degree and frequency of this response. Indeed, the cytologic method of radiosensitivity testing has been criticized because of the influence of age and hormonal status.

Observations based on studies of both vaginal biopsies and exfoliative cytology have demonstrated that radiation changes occur most often in postmenopausal women, although the prognostic value is greater in the premenopausal group. Among the patients being studied in our laboratory, the incidence of good RR is definitely greater in the postmenopausal group. Of 45 postmenopausal women the RR was good in 44 (98%); whereas, in a group of 32 premenopausal women the RR was good in 19 (59%). Moreover, this difference in radiation response appears to be related to age, as well as to degree of ovarian activity; for in our cases there is a straight-line increase in incidence of good RR with advancing age, as indicated by the following breakdown according to age:

22 - 39 years of age	--	19 patients	--	12 with good RR
40 - 49 " " "	--	15 patients	--	11 " " "
50 - 59 " " "	--	15 patients	--	13 " " "
60 - 69 " " "	--	15 patients	--	All " " "
70 years plus		13 patients	--	All " " "

Not only does the frequency of good RR increase with advancing age, but the actual count is generally higher in the older patients.

It is our impression that supervoltage radiation produces a good cytological response with unusual frequency. A few selected patients with carcinoma of the cervix have been treated, on an experimental basis, by a 70 million volt synchrotron. These patients, selected because of advanced disease or tumors anatomically unfavorable for radium therapy, have usually received a diffuse pelvic dose of 6000 r. Sixteen patients have been studied. Exactly one-half have died or have recurrent malignant disease. However, all showed a good cytologic radiation response. Moreover, the curve of serial counts generally obtained a higher level and rose faster than those receiving external radiation with the more conventional supervoltage radiation by the 1000 kv machine. Such observations on a small group of patients are presented to stimulate further work. We are aware of the contrary opinions.

The former observations may be explained, in part, by age and the fact that patients selected for treatment with the synchrotron generally have far-advanced disease; for in our experience, good RR is associated more frequently with advanced tumor than early tumor. Thus, of 56 patients with Stage I and Stage II carcinoma of the cervix, 43 (77%) had good RR. Of 11 patients with Stage III or Stage IV carcinoma of the cervix, ten (91%) had good RR. The number of cases is too small to eliminate the interrelated influence of age and menopausal status.

The growth characteristics of the cervical tumor do not appear to influence significantly the degree of radiation response, although good RR was associated with an endophytic growth slightly more often than with an exophytic growth.

Moreover, the patient's general health, as evidenced by weight loss, anemia, bleeding during therapy, or obesity, does not significantly influence radiation response. Obese patients had a very slightly higher incidence of good RR: 28 of 29 obese patients showed good RR while 34 of 43 non-obese patients had good RR.

It may be noted parenthetically that six patients who were noted to have severe skin or other radiation reactions during therapy showed a good radiation response.

SUMMARY

Based on this admittedly limited experience, we conclude that cytologic radiation response is influenced by age and menstrual status. Good radiation response is more frequent in the postmenopausal

*From the Department of Obstetrics and Gynecology and the Cancer Research Institute, University of California, School of Medicine, San Francisco, California, U.S.A.

women and increases with advancing age. Possibly related to this, patients with advanced clinical extent of disease frequently demonstrate a good RR. Growth characteristics of the tumor and general health of the patient appear to have little influence upon the cytologic radiation response. We seriously doubt the validity of experiments utilizing vitamins or hormones to improve RR. It is our impression, not yet confirmed by adequate data, that supervoltage radiation (in the experimental range of 70 million volts) produces more frequent and intense radiation response than conventional x-ray therapy.

These observations have a direct bearing on the value of cytology as a means of prognosticating response to radiation therapy.

Bibliography

1. Merrill, James A.: Progress in Radiation Therapy. New York, 1958, Grune and Stratton.

DISCUSSION

JORGE CAMPOS R. de C., Lima, Peru:

The observations of Merrill and Wood and Kjellgren both point out that a good cytological response is more frequent among postmenopausal women and among women with poor hormonal levels and that the less favorable responses are common in premenopausal patients with high hormonal levels.

From here we can assume that one of the most important factors in determining the response of the host and the tumor during treatment is the estrogenic stimulus. In this way carcinoma of the cervix may be considered a hormonal dependent.

A factor which must be investigated more deeply is the effect of supervoltage in radiation response.

CLOSING REMARKS

JAMES A. MERRILL and DAVID A. WOOD:

It is very interesting and of definite significance that in our rather small series the relationship between age and menopausal status and cytologic response was the same as noted in the much larger series of Kjellgren. The frequent occurrence of a good response associated with the advanced stages of disease and with supervoltage radiation seems real but these too may be related to age and menopausal status. The increased frequency of marked radiation injury in patients with good cytologic response noted by us and by Kjellgren is probably related to greater systemic radiation sensitivity of these patients.

Campos' conclusions regarding possible hormone dependency of cervical carcinoma do not seem warranted from these data. Certainly there is a relationship between the changes noted in normal exfoliated vaginal cells following radiation and the patient's age and estrogenic activity. However, this does not offer convincing or even suggestive evidence that the growth and/or response of cervical carcinoma is related to these factors. Indeed, there is evidence to the contrary. We must be cautious about allowing our zeal over laboratory techniques to lead us down the path to hasty or inaccurate conclusions.

CYTOLOGICAL RESPONSE AS COMPARED WITH LOCAL PRIMARY HEALING AND WITH LOCAL RECURRENCE AND METASTASES OF CERVICAL CARCINOMA

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Gothenburg, Sweden

The cytological response was compared with local primary healing and with local recurrence and metastases of cervical carcinoma in 287 patients.

Local primary healing: The criteria adopted in the present investigation for local primary healing were that tumor lesions no longer should be encountered at clinical examination of the uterine cervix and upper third of the vagina and that the cervix should feel normal or atrophic on palpation. The results of the analysis are shown in Table I and Figure 1.

The preponderance in the rate of primary local healing failure in the poor response group compared with the good response group is highly significant ($\chi^2 = 34.325$, $df = 1$, $p < 0.001$). The difference in local primary healing rate between the poor and good response groups was highly significant for Stage II ($\chi^2 = 14.619$, $df = 1$, $p < 0.001$) and significant for Stage III ($\chi^2 = 10.147$, $df = 1$, $0.01 > p > 0.001$). Within the poor response groups the difference in local primary healing rate between Stages I and III was highly significant ($\chi^2 = 12.323$, $df = 1$, $p < 0.001$) and between Stages I and II nearly significant ($\chi^2 = 4.197$, $df = 1$, $0.05 > p > 0.02$).

TABLE I

	Poor response	Good response	Total
Stage I	10.6 ± 4.5 %	0 %	5.2 ± 2.2 %
Stage II	26.1 ± 5.3 %	1.5 ± 1.5 %	14.2 ± 3.0 %
Stage III	46.4 ± 9.4 %	4.0 ± 3.0 %	26.4 ± 6.5 %
Stage IV	75.0 ± 21.6 %	—	75.0 ± 21.6 %
Total	26.4 ± 3.6 %	1.4 ± 1.0 %	14.3 ± 2.1 %

Frequencies of primary healing failure in Stages I to IV within the poor and good response groups.

Local recurrence: For the purposes of the present investigation a local recurrence was defined as the development of a clinically manifest tumor on the cervix or in the upper third of the vagina, if on any previous occasion after the end of treatment the uterine cervix had been described as normal or atrophic. Local recurrence rates in the various stages of the two cytological response groups are given in Table II and Figure 2. The local recurrence rate in the poor response group showed a highly significant excess over that in the good response group ($\chi^2 = 18.099$, $df = 1$, $p < 0.001$). The difference between the poor and good response groups in local recurrence rate was nearly significant for Stage I ($\chi^2 = 5.400$, $df = 1$, $0.05 > p > 0.02$) and significant for Stage II ($\chi^2 = 7.595$, $df = 1$, $0.01 > p > 0.001$). Within the poor response group there were no differences of significance between the various stages.

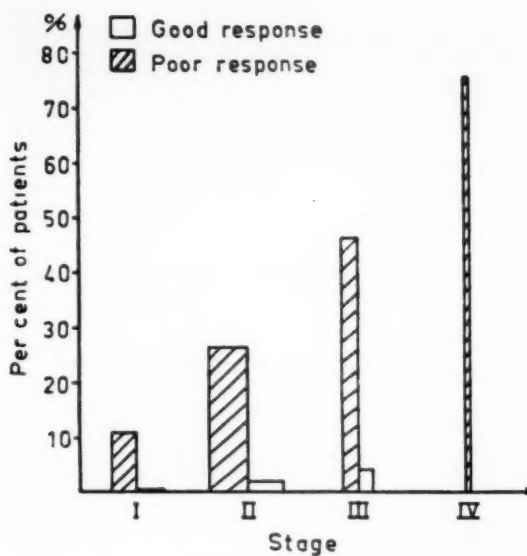


Fig. 1. Frequencies of primary healing failures in Stages I to IV within the poor and good response groups.

TABLE II

	Poor response	Good response	Total
Stage I	$28.1 \pm 7.9 \%$	$6.4 \pm 3.6 \%$	$15.2 \pm 4.0 \%$
Stage II	$29.5 \pm 6.9 \%$	$6.0 \pm 3.4 \%$	$17.0 \pm 3.9 \%$
Stage III	$35.7 \pm 12.8 \%$	$12.5 \pm 8.3 \%$	$23.3 \pm 7.7 \%$
Stage IV	0 %	—	0 %
Total	$29.7 \pm 4.8 \%$	$7.1 \pm 2.4 \%$	$17.2 \pm 2.8 \%$

Local recurrence rates in Stages I to IV within the poor and good response groups after previous local primary healing.

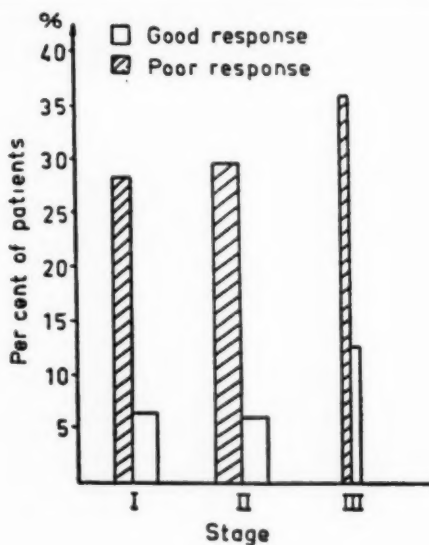


Fig. 2. Local recurrence rates in Stages I to III within the poor and good response groups after previous local primary healing.

Metastases: The clinical metastasis frequency, whether in the form of spread to pelvic or remote lymph nodes, to distant organs or to the lungs, was calculated for patients without locally persistent or recurrent cancer. It appeared that the metastasis frequency was the same for the poor and good total response groups ($17.5 \pm 3.4\%$ and $17.4 \pm 3.4\%$). Accordingly, there was no evidence of a higher incidence of metastases among locally healed patients with poor responses than among similar patients with good responses.

Bibliography

1. Kjellgren, O.: Acta Radiol. Suppl. 168, 1958.

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COMPARISON OF CELLULAR RADIATION CHANGES IN PATIENTS WITH CARCINOMA OF THE CERVIX AND TUMORS OF THE HEAD AND NECK AREAS

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This is a preliminary qualitative cytologic study of radiation changes of squamous epithelium in patients with a tumor of the head and neck area and patients with carcinoma of the cervix.

Material and Method

Fifteen cases were selected in each group on the basis of the following criteria:

- 1 No history of previous radiation.
- 2 Uniform type of radiation therapy.
- 3 Approximately the same amount of radiation.

All patients in the head and neck group received radiation by means of the betatron (electron beam), and all patients in the group with carcinoma of the cervix received radiation by means of a one million electron volt unit (1 MEV).

The patients in the head and neck group consisted of fourteen males and one female, ranging in age from 47 to 69 years. Eight patients were treated for carcinoma of the tongue, one for carcinoma of the tonsil, one for reticulum cell sarcoma of the tonsil, one for carcinoma of the pharynx, one for carcinoma of the soft palate and one for carcinoma of the larynx. Seven of these patients had lymph node metastases. Two additional patients were treated for metastatic cervical lymph nodes with a primary suspected in the nasopharynx.

In the group with carcinoma of the cervix the ages ranged from 38 to 75 years. Fourteen of the cases were diagnosed as epidermoid carcinoma and one case as carcinoma of Gärtner duct origin. Seven patients had surgery prior to radiation and were classified as Stage III. All had recurrent pelvic disease at the time of radiation. Of the remaining eight patients none had previous surgery. Four patients were classified as Stage II and three as Stage IV. One was considered as inoperable at the time of admission.

In the head and neck group routine smears were obtained from the primary site of the tumor and from the vallecule which was found to be a pool for exfoliated cells from the oral cavity.

In the group with carcinoma of the cervix, cervical and vaginal smears were obtained in patients who still had their cervixes; vaginal smears only were taken from patients in whom the cervix was surgically removed.

Seventy-five smears with an average of five smears per patient were examined in the gynecological group; ninety-five smears with an average of six smears per patient were examined in the head and neck group. Attention was paid to radiation changes in benign cells only. The criteria for radiation changes were based on Ruth Graham's observations and are enumerated as follows:

*From the Strang Laboratory of Cytology, Memorial Center for Cancer and Allied Diseases, New York, New York. This work has been supported by a Grant from the National Cancer Institute.

I. Superficial and intermediate cells:

A. Cytoplasmic changes

Enlargement

B. Nuclear changes

Enlargement

Multinucleation

II. Parabasal and basal Cells:

A. Cytoplasmic changes

Enlargement

Vacuolization (multiple)

B. Nuclear changes

Enlargement

Multinucleation

Scanty smears were eliminated. The entire smear was screened and the changes described above were recorded for each cell.

In order to obtain comparable data, the patients were grouped according to the amount of radiation at a given time: at approximately 1000, 2000, 3000, and 4000 r.

Results

After computation of results it became evident that the behavior of the parabasal cells in both groups was not significantly different.

However, there was a significant difference in behavior of the superficial squamous cells (Table I).

TABLE I

CYTOPLASMIC CHANGES			NUCLEAR CHANGES	
	ENLARGEMENT	VACUOLIZATION	ENLARGEMENT	MULTINUCLEATION
1000 r				
Head & Neck No. of Pts.	13	2	15	10
Ca of Cx No. of Pts.	6	1	9	2
2000 r				
Head & Neck No. of Pts.	14	5	15	12
Ca of Cx No. of Pts.	11	1	9	7
3000 r				
Head & Neck No. of Pts.	13	4	14	8
Ca of Cx No. of Pts.	10	0	13	9
4000 r				
Head & Neck No. of Pts.	13	4	12	10
Ca of Cx No. of Pts.	12	0	12	10

At 1000 r cellular enlargement, multinucleation and nuclear enlargement of the superficial cells were more prominent in the head and neck group than in the gynecological group.

At 2000 r the difference in enlargement of superficial cells was not appreciable; however, multinucleation and nuclear enlargement were still greater in the head and neck group. In addition, vacuolization of the cytoplasm was more pronounced among the head and neck patients.

At 3000 and 4000 r the smear patterns were essentially the same in both groups except for a greater number of cells with cytoplasmic vacuolization among the head and neck patients.

Discussion of the Above Results

The above results suggest that the squamous epithelium of the mouth displays radiation changes earlier than the squamous epithelium of the gynecological tract. This difference is in all probability due to the use of a more penetrating source of radiation and to the anatomical setting, since the radiation of cervical cancer with the 1 MEV machine is done through abdominal ports.

Enlargement of superficial cells appears to be the earliest manifestation of radiation changes. Of interest is the late occurrence of cytoplasmic vacuolization of the superficial cells. This appears to be a degenerative phenomenon, secondary to other changes in superficial cells.

The lack of differences in behavior of parabasal cells in the two groups is puzzling and suggests that perhaps these cells are not readily influenced by radiation and that the changes observed may be due to other factors, such as inflammation. However, we do feel that our series is too small to draw any definite conclusion.

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COMPARISON OF RADIATION CELL CHANGES IN EXFOLIATED VAGINAL CELLS AND IN EXFOLIATED CELLS FROM THE URINARY TRACT (UROCYTOGRAM)

PIERRE HAOUR
Lyon, France

As it has already been shown in many publications on urinary smears, there is a great analogy of cellular morphology and also a close parallelism of hormonal changes when vaginal exfoliation is compared with cells of the urinary sediment (urocytogram) (1).

In cancers treated by radium or x-ray, the bladder epithelium receiving radiation exhibits characteristic changes (2). Therefore, exfoliated cells in the urine sediment may also show morphological alterations. This is a preliminary report about the urocytogram during and after radium therapy.

We have been following by repeated smears 40 patients who received radium therapy for cancer of the cervix; we found characteristic Radiation Response (RR) cells in the urocytograms of 36 patients.

The changes observed were nearly similar to the modifications described in vaginal smears:

I. Increase in size is one of the main features noticed in the irradiated urinary cells (Fig. 1, 2).

The figures found by measuring cells are far above the mean values of normal urinary cells (Figs. 3, 4), some cells being over 100 in diameter. This change is especially evident in intermediate cells.

As compared with vaginal smears, when one considers that normal urinary cells are much smaller than vaginal cells, the increase in size is more marked in the urocytogram (Fig. 4).

Abnormally shaped cytoplasm is also evident (elongated cytoplasm, plasmodial pictures). Though multinucleation is often encountered, nuclear diameters do not increase in the same proportions as the cytoplasm. Vacuolated cells are not frequent; polynuclear cells appear in all cases from the beginning of treatment.



Fig. 1. Vaginal superficial cells four days after the first radium application. RR cells-increased size as compared with normal cells.

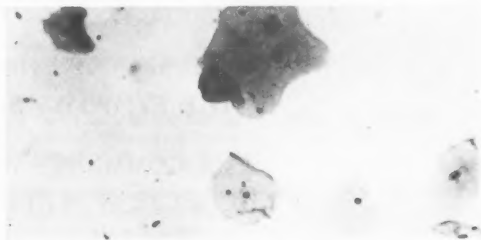


Fig. 2. Corresponding urinary cells simultaneously obtained.

NON-IRRADIATED		IRRADIATED	
Int. cells	Sup. cells	Int. cells	Sup. cells
34	36.6	75.8	75.7

Fig. 3. Urinary cell size in μ (intermediate and superficial cells) in non-irradiated and irradiated cases.

VAGINAL SMEARS		URINARY SEDIMENT	
Int. cells	Sup. cells	Int. cells	Sup. cells
73.0	64.9	62.4	60.5
(55.1 - 107.7)		(46.6 - 95)	

Fig. 4. Cellular size in μ (intermediate and superficial cells) in vaginal smear and urinary sediment after irradiation.

II. RR cells are seen as early as the second day of radium therapy and increase in number after the second radium application.

The urocytogram is more advantageous than the vaginal smear in following the early effects of radiation, even during radium application. Necrotic alterations are not as common in the urinary sediment as in vaginal smears after radium has been removed.

The urocytogram does not give, of course, any information concerning the intensity of response of cancer cells, but the method yields information about the response of normal cells and about the hormonal effect during radiation therapy (evaluation of Karyopyknotic Index).

Until now we have not been able to compare a large enough number of cases. Thus, it is too early to appreciate the practical value of the method as a cytological procedure for determining the prognosis.

Bibliography

1. Del Castillo, E.B., Argonz, J. and Galli-Mainini, C.: J. Clin. Endocrinol. 8:76, 1948.
2. LaCassagne, A. and Gricouroff, G.: Action des radiations ionisants sur l'organisme. Paris, 1956, Masson.

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RADIATION CELL CHANGES IN CELLS FROM THE ORAL CAVITY

JACQUELINE MOURIQUAND, MARCEL DARGENT AND JEAN PAPILLON

Lyon, France

Interpretation of smears after x-ray therapy presents great difficulty to the cytologists. Our experience is based on 550 cytological examinations of oral diseases collected since 1953. One hundred and ninety-four of them were made after radium, cobalt or x-ray therapy. The results have led us to be very cautious in the interpretation of the smears.

Most of these cases have been followed every three months since the termination of treatment. In some cases five or six different cytological examinations were necessary before disappearance of the radiation cell changes occurred.

Those changes, observed in the oral cavity, are seen only in the superficial and intermediate cells; parabasal cells are infrequently encountered (1, 2, 3, 4, 5, 6, 7, 8). These alterations are to a certain extent comparable to the description of Ruth Graham (9, 10, 11, 12) concerning vaginal smears. A few differences, however, are to be seen.

Radiation changes in benign cells: A remarkable enlargement of the cell may be found, but this is exceptional. One may find four or five cells of this type in the most favorable cases and then only during treatment.

Slight increase of the cell surface with simultaneous enlargement of the nucleus is often seen: normal cell diameter is approximately 50μ in superficial squamous cells of the oral cavity. It may increase to 65 or 70μ at the most (Figs. 1 and 2).

Cells with two or more nuclei are often seen. Vacuoles in the cytoplasm are infrequent and may be seen in inflammation and cancer; therefore, their presence is not characteristic. Bizarre forms are exceptional.

The percentage of benign cells altered by x-ray therapy is much lower than those described in vaginal cells. Only 20 to 30% of oral cells will show the above signs. Therefore, we do not take into account the percentage of radiated cells, but we evaluate the mean cell diameter, a method we think is more to the point.

Radiation changes in malignant cells: Remarkable nuclear monstrosities or sometimes a characteristic hydropic aspect of the nucleus are observed. The nuclei and nucleoli are both increased in size. Using methylgreen - pyronin stain, one can see these voluminous nucleoli stained red (ribonucleic acid: RNA), while the green staining of chromatin (desoxyribonucleic acid: DNA) seems diminished when compared to malignant cells (13, 14, 15).

In some of our smears both radiated malignant cells and intact malignant cells are present, as evidenced by clinical recurrence. Accurate distinction between these two types of cells seems to us impossible with the Papanicolaou staining technique (16, 17) (Figs. 1, 2, 3, 4, 5, 6).

One of our patients was submitted to 9800 r for a spindle cell carcinoma of the left tonsil. Cytology performed seven months after treatment revealed nuclear monstrosities such as may be seen under x-ray therapy. Methylgreen - pyronin stain showed them to be rich in DNA. On this basis we concluded it was an active malignancy, which was confirmed by further development of the tumor.

From the Institut Pasteur and Centre Leon Bérard of Lyon, Lyon, Rhone, France.

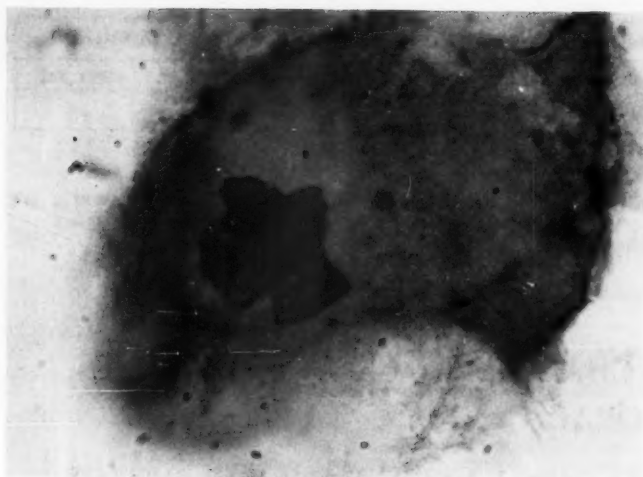


Fig. 1

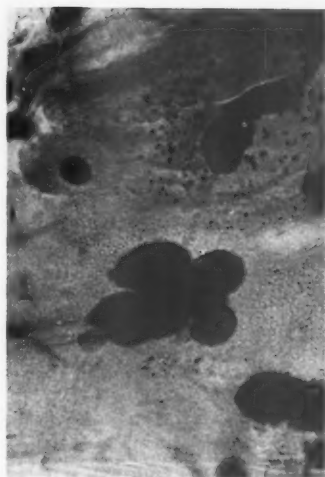


Fig. 2

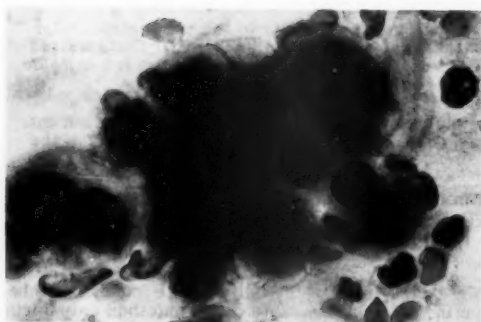


Fig. 3

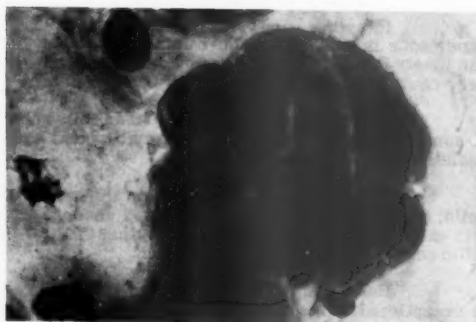


Fig. 4

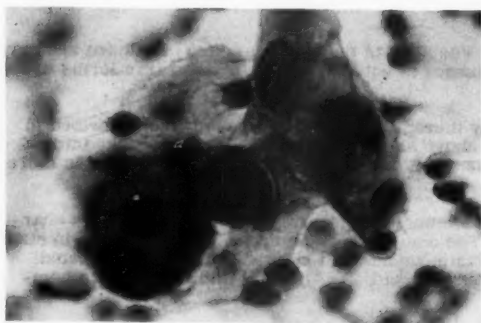


Fig. 5

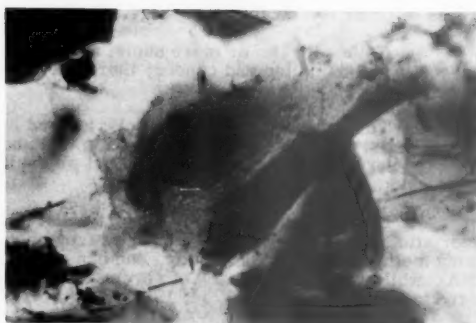


Fig. 6

Histophotometric studies of possible differences in the DNA content of the nuclei of active malignant cells and radiated cells would be desirable.

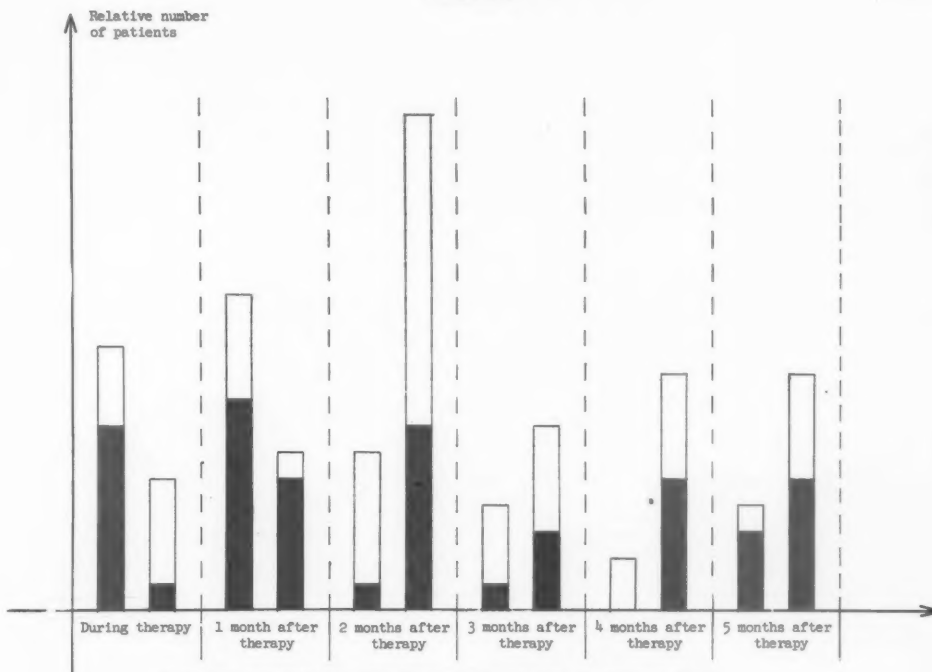
The duration of radiation alterations is highly variable. In some cases repeat smears one month after radium treatment disclosed only normal cells (18). In others, these normal cells were found only three or four months after treatment. One of our patients still had very marked alterations eleven months after therapy.

Extreme variations in these disorders following irradiation in the months following treatment with radium, cobalt or x-rays has led us to search for a possible relationship between the cytological patterns and the response of the tumor to x-rays.

Table I sums up our cases and demonstrates:

1. from the first month on, a regular decrease with time of the radiation cell changes with, however, a temporary increase at the fifth month that we will discuss later.
2. no relationship between the clinical evolution and the existence of cellular response to treatment, in the months following treatment.

TABLE I



Distribution of Radiation Cell Changes Compared with the Clinical Course

In each monthly section the left column represents the total number of cytological examinations containing radiation cell changes. The column at the right represents the number of cytological examinations without radiation cell changes. The clear area of each column shows the favorable clinical courses, the dark area represents those cases in which clinical recurrence soon occurs. All cases have been followed up over a period of one year.

One of our patients presenting very important alterations one month after x-ray therapy died nine months later from recurrence of the tumor. Another one treated by cobalt (6600r/T) for a squamous cell carcinoma of the tongue showed no cellular disorders at the end of treatment (mean cellular diameter: 53.75μ) and is still cured one year later.

Cytology was performed in 15 cases during treatment or immediately afterwards and also did not show any relationship between the importance of radiation cell changes and the tumor response to x-rays.

We have been particularly concerned with the evaluation of the mean cellular diameter during x-ray therapy. Two cytological examinations per week were done as long as treatment lasted. Lack of sufficiently long follow-up does not allow any mentioning at the moment.

Several of our patients who were seen in the months following radium therapy had an ulcerated lesion for which clinical examination was indeterminate between radionecrosis and recurrence. Cytology was a great help here (19). Intermediate cells of normal size were seen with generally nucleolated nuclei and finely granular chromatin. In other words, no cytologic signs were seen, either of malignancy or of typical radiation cell changes. Whenever recurrence is present, characteristic modifications of malignancy are present.

We shall now describe a second type of cellular anomaly observed much later, from the third or fourth month on, after x-ray therapy. The smears contain a majority of intermediate cells with nucleolated nuclei and reticular chromatin with an active aspect. Intracytoplasmic vacuoles may occasionally be seen. Other cells are of the superficial type with an orange cytoplasm, and the nuclei are often hyperchromatic. No increase in cell size is present.

When one keeps in mind the lack of nuclear or cellular monstrosities in some very orthoplastic spindle cell carcinomas of the oral cavity, one understands the cytologist's concern. Diagnosis is based especially upon the abundance of these intermediate nucleolated elements that constituted, in a rather homogenous way, all of the cellular population.

This type of late x-ray radiation change may be encountered more than one year after treatment. It does not bear any relation to the evolution of the disease any more than the typical radiation changes do, at least as far as the cases we have met are concerned.

Summary and Conclusions

Our experience concerning 194 cytological examinations of the oral cavity after irradiation has shown us that the cellular changes do not exactly reproduce those observed in vaginal cells. In our cases the importance of these x-ray therapy modifications appears less and seems unrelated to the response of the tumor to treatment. We think it is not possible to make any distinction between active malignant cells of certain types and radiated malignant cells, at least with Papanicolaou's method. Staining with methylgreen - pyronin was a help in some cases, showing a decrease of the nuclear DNA of the irradiated malignant cell.

However tempting it may be to search for a relationship between by physiotherapy induced cellular modifications and response of the tumor to treatment, one may question its value (20). These alterations are evaluated chiefly on benign cells that may have a different behavior than tumor cells, whose responsiveness one is trying to evaluate.

Bibliography

1. Morrison, L. F., Hopp, E. S. and Wu, R.: *Annals of Oto. Rhino. and Laryng.* 58:18, 1949.
2. Miller, S. C., Soberman, A. and Stahl, S. S.: *Journ. Dent. Research* 30:4, 1951.
3. Weinmann, J. P.: *J. Dent. Res.* 19:57, 1940.
4. Montgomery, P. W.: *J. Dent. Res.* 30:12, 1951.
5. Montgomery, P. and Von Haam: *J. Dent. Res.* 30:260, 1951.
6. Montgomery, P. and Von Haam: *J. Dent. Res.* 30:308, 1951.
7. Peters, H.: *Am. J. Clin. Path.* 29:219, 1958.
8. Dargent, M., Papillon, J. and Mouriquand, J.: *Bulletin du Cancer* 42:215, 1955.
9. Graham, R.: *Surg. Gyn. and Obst.* 84:153, 1947.
10. Graham, R.: *Surg. Gyn. and Obst.* 84:166, 1947.
11. Graham, R. and Graham, J. B.: *Cancer* 6:215, 1953.
12. Graham, R.: *The Cytologic Diagnosis of Cancer.* Philadelphia, 1950, Saunders.
13. Lison, L. F.: *Histochimie et Cytochimie animales.* Paris, 1953, Cauthier-Villars.
14. Garces, B.: *Bulletin du Cancer* 42:215.
15. Kritter, H. and Herovici, C.: *Bulletin du Cancer* 43:513, 1956.
16. Sicard, A. and Marsan, C.: *Presse Médicale* 62:245, 1954.
17. Menasche, C.: *Surveillance par les frottis vaginaux des malades traités pour Cancer utérin.* Paris, 1953, Thèse.
18. Hopp, E. S.: *The Laryngoscope* 68:1281, 1958.
19. Silverman, S., Beck, H. and Farber, S. M.: *J. Dent. Research* 37:195, 1958.
20. Wachtel, E.: *Revue Française de Gynécologie et Obstétrique* 53:653, 1958.

HANNAH PETERS

Copenhagen, Denmark

Cancer of the mouth treated by radiation offers a convenient opportunity to study progressive cell changes caused by radiation in different parts of the same epithelium. During x-ray therapy different areas of the mouth receive radiation: (1) the area of the tumor for which the therapy is specifically given, (2) the tissue which immediately surrounds the cancer and (3) parts of the normal epithelium. If smears are taken from these three defined areas under direct vision at frequent intervals during therapy, changes induced by radiation can be followed in cells whose anatomical origin is known. We shall be concerned here only with the changes observed in cells obtained from normal epithelium and compare them with changes found in the epithelium surrounding a cancer.

The normal epithelium of the mouth consists of stratified squamous epithelium and smears taken from a normal buccal mucosa show mainly large superficial squamous cells (Fig. 1). They are 60 to 75 μ in diameter; only an occasional one may reach 100 μ . Their cytoplasm is transparent. An occasional cell contains a few cytoplasmatic basophilic granules, which are unevenly scattered throughout the cyto-

plasm. In non-irradiated cells these round bodies are usually smaller than 2μ . Cells with one or two such granules are not infrequent, but cells containing five or more are rare.

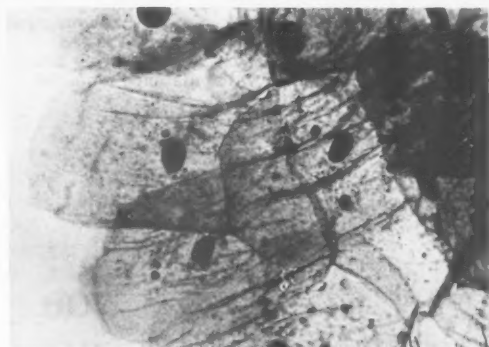


Fig. 1. Large superficial squamous cells.
Smear taken from a normal buccal mucosa.

Smears taken during therapy from the normal epithelium reveal changes in the squamous cells which began to appear soon after radiation is started. The appearance of many cells with large numbers of cytoplasmic granules is most characteristic (Fig. 2). In the beginning of therapy, cells containing 10 or 20 granules are seen, but this number increases rapidly to a peak at about 14 days after therapy has been started, when cells with 60 to 90 granules are common. At this time 90% of all squamous cells seen on the slide may show this change. The granules are usually larger than 2μ ; some indeed may be considerably larger, reaching the size of 5μ . The granules stain red with methyl green-pyronin but fail to stain after ribonuclease digestion, suggesting that they contain ribonucleic acid. The appearance of the cytoplasmic granules is the most constant radiation induced change observed in the normal squamous cell of the mouth. Other changes are occasionally noted: enlargement of the cell occurs, but is infrequent. In most cells the cytoplasm remains transparent, but at times one with opaque cytoplasm will be found. Nuclear changes are rare, an occasional cell with two nuclei may be seen, but the size, shape and appearance are usually unaffected. The cell borders remain sharply defined and clean cut.

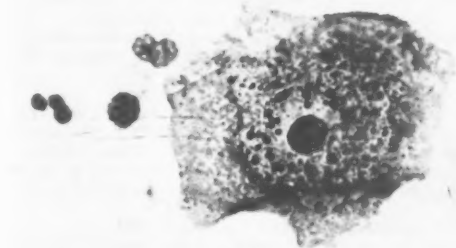


Fig. 2. Large superficial squamous cell containing many cytoplasmic granules.
Smear taken from normal epithelium during radiation.

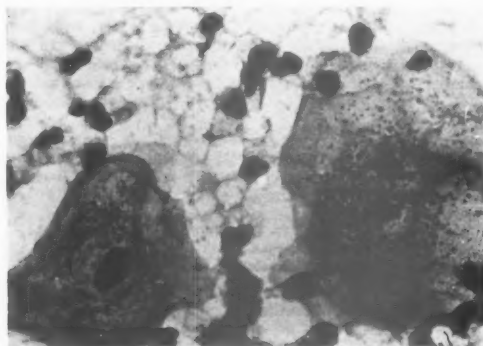


Fig. 3. Cells obtained from an area surrounding a cancer show marked changes during radiation therapy.

Smears obtained before radiation are taken from epithelium close to a cancer or surrounding it and are similar to those smears taken prior to radiation from the normal part of the epithelium some distance away from the lesion. The squamous cells obtained from areas close to a cancer are morphologically not distinguishable from squamous cells taken from normal epithelium. However, soon after radiation has begun, smears taken from areas close to a cancer show different cell reactions than those smears from a normal though radiated epithelium. The smears, in addition to cells with cytoplasmic granules, begin to show a marked pleomorphism early during radiation therapy (Fig. 3). Marked variation in the size of the cells is noted, many reaching 110μ and more. Odd and bizarre cell shapes, almost never seen in radiated cells from normal epithelium, become characteristic in smears taken from the area close to the cancer. Changes in the staining reaction of the cytoplasm is characteristic; opaque cytoplasm as well as intense eosinophilic staining is conspicuous. Perinuclear vacuoles, as well as multiple vacuolization and streaking of the cytoplasm, appear. The cellular outlines, which remain so well defined in radiated normal squamous cells, become indistinct and hazy. The nuclear reactions are equally varied and manifold. Cells with enlarged and bizarre nuclei are commonly seen in the cell population; chromatin clumping and large nucleoli are frequent. Double, as well as multiple nuclei, in bizarre or enlarged cells are characteristic.

Summary

Though no morphological difference can be noted before radiation is started, a distinct difference in the radiation response has been observed in epithelial cells growing close to a cancer as compared to the cell reaction in the healthy parent tissue. The reaction to radiation appears to be uniform and slight in the normal cells and manifold and marked in cells originating close to a malignancy. This sensitivity to radiation of the squamous epithelial cell increases with the decrease of the distance from the frankly malignant tissue.

Bibliography

1. Peters, H.: Am. J. Clin. Path. 29:219, 1958.
2. Peters, H.: Second U.N. Intern. Conf. on the Peaceful Uses of Atomic Energy, Geneva, 1958 (in press).

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HOW SOON AFTER THE BEGINNING OF THERAPY CAN THE RADIATION RESPONSE BE JUDGED ACCURATELY?

OLLE KJELLGREN

Gothenburg, Sweden

In order to assess the influence of the time factor on the radiation reaction in irradiated cervical carcinoma, smears were obtained every two or three days from each of 46 patients during the period beginning on the day of the first radium application and ending about two months later. Charts in which the percentage of radiation cell changes are plotted against time elapsed after the first radium application will be found for each individual patient in my book: "The Radiation Reaction in the Vaginal Smear and Its Prognostic Significance."

The investigation showed that the percentage of epithelial cells with radiation changes in the vaginal smear began to rise after a latent period of a few days following the first radium application. It peaked shortly before or after the second radium application (about two weeks after the first application). In some patients the curve of the percentage of radiation cell changes had no real peak; it merely rose steadily to a high level. In other words, there was no culmination and the signs of radiation reaction in the smears persisted.

Some curves rose steeply to the good response level, i.e., 60% or more; others fluctuated in the low response region. The peak response to the first radium application occurred after 11.88 ± 0.47 days with a dispersion about the mean of 2.99 ± 0.33 days. The degree of the radiation reaction in the vaginal smear tended to drop slightly on the day of or shortly after the second radium application, whereupon it started to rise once again. The peak response to the second radium application occurred after 10.5 ± 0.30 days with a dispersion of 1.94 ± 0.20 days about the mean.

The maximum radiation reaction after a radium application, thus, should be expected after some 10 to 13 days. At this time the degree of the radiation reaction is, as a rule, representative. For example, in the investigation discussed above, the type of radiation reaction in the smear obtained immediately before the second radium application, about two weeks after the first, could be told in $91.3 \pm 4.2\%$ of the patients.

Figure 1 shows the mean cytological response up to the second radium application, plotted against time after the first radium application, for those patients whose vaginal smears showed a frequency of radiation cell changes of 60% or better at least once and for those patients who had a poor cytological response.

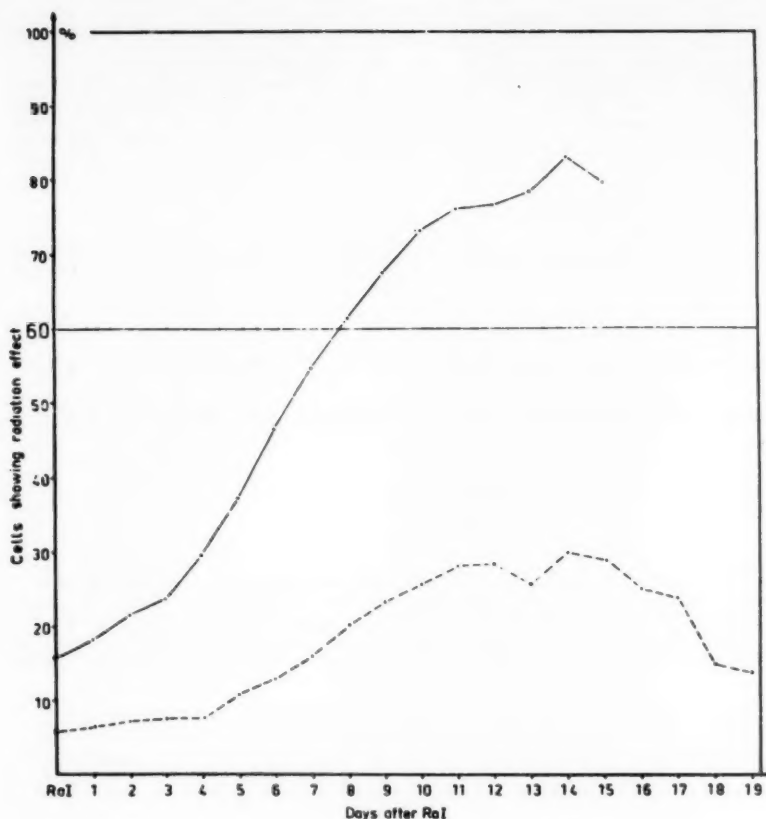


Fig. 1. Configuration of curves for mean poor and good response after Ra I.

Bibliography

1. Kjellgren, O.: Acta Radiol. Suppl. 168, 1958.

DISCUSSION

D. A. BOYES, Vancouver, British Columbia, Canada:

This is an important aspect in the study of radiation response, and in our hands it does not appear to be as clear as it was in Ruth Graham's study or in Kjellgren's study. For reasons of convenience to our patients, most of whom are outpatients, smears were taken approximately one week after the first insertion of radium (modified Manchester Technique), two weeks after the second, and one week after the third insertion. Smears are also taken half way through the x-ray therapy and at the termination of x-ray therapy. Thirty-five of the smears became "good" after the first insertion, 42% after the second and 22% after the third insertion of radium. In addition, there are several cases whose response did not reach the good level until they were half way through their x-ray therapy. Whether these differences in the various centers represent an effect of different mode of therapy or whether the workers involved are using the technique with some individual variation is difficult to know. If subsequent adjuvant therapy were contemplated in our patients, it could not be applied until after completion of three insertions of radium, and even then a small and as yet unknown percentage would receive unnecessary adjuvant therapy as they would have produced a good radiation response on their own.

ALFRED GLÜCKSMANN, Cambridge, England, U.K.:

Histological observations on changes in the tumor cell population fully support Kjellgren's cytological findings. An increase in the proportion of differentiation (keratinizing) cells may be noted within one day of starting the radium treatment and a peak value of about 60% may be reached by the seventh day in cases responding favorably to treatment (Curve A in Figure 1). Cases with a poor response (Curve C) present only minor fluctuations in the incidence of differentiating cells and cases with a partial and ulti-

mately unsatisfactory response (Curve B) show only a tardy rise to no more than 40%. The percentage count of viable cells drops corresponding to the rise in the differentiating cell count.

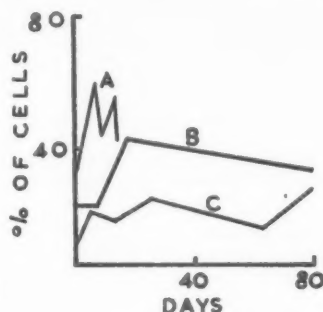


Fig. 1. Percentage changes in the incidence of keratinizing cells in cervical cancers following radium treatment.

Kjellgren's figures for cells showing RR closely resemble our data for differentiating cells (see his graph and ours). Since the histological observations are made on cells prior to their exfoliation, the cellular changes are noticeable slightly earlier than those made in smears. The speed of the radiation reaction is influenced by dosage and big doses of radium tend to delay their appearance.

EMMERICH von HAAM, Columbus, Ohio, U.S.A.:

By placing the two applications of radium two weeks apart, Kjellgren was able to determine in over 90 per cent of his patients the expected radiation response before the second radiation treatment was applied. This would make it possible to change the method of therapy in those patients in whom a poor radiation response was obtained.

PIERRE HAOUR, Lyon, France:

For those who cannot follow patients by repeated smears after irradiation, it appears quite practical to have a standard time after irradiation during which the greatest number of optimum responses might be found. Ruth Graham has already given this time as being from nine to 16 days, (in a preceding paper in this Symposium). This differs a little from Kjellgren's figures (10 to 13 days after radium application) - this time, if I have understood it correctly, being after either the first or the second radium application.

The first results of our observations of irradiated urinary cytology also show the majority of good responses as being between these limits. The mean value of the highest percentage found was not over 50% (only increased size being considered).

HORST SMOLKA, Kiel, Germany:

The radiation technique used on cervical carcinoma during our cytological examinations consisted of three radium applications with a single dosage of 2000 mgh (50 mg for 40 hours) at intervals of two weeks each, followed by fractionated x-ray radiation. With this kind of therapy the first radiation changes occurred in single cases already in the third or fourth day of radium application, the latest appearance being on the tenth day. In 50% of the cases the changes could be recognized during the first week. A specific day where the first changes appeared, in the majority of cases, could not be affirmed.

No statements can be made as to the number of cells which disclose radiation changes at the moment of their first appearance. The reason is that one cannot on a given slide determine how many cells really stem from the irradiated part of the vagina and how many originate from other non-irradiated parts of the vagina. That the latter display only little if any radiation changes is understandable because of the abrupt decrease of the radiation dose. As a consequence of the various factors upon which the composition of the cytological specimen depends, the number of cells displaying irradiation changes does not give a clue as to the number of cells in-situ which have been hit by radiation. After the appearance of the first altered cells, the number of cells with changes increases rapidly. However, during the following period the amount of such cells fluctuates considerably, depending upon the site from where the specimen is obtained, the amount of vaginal secretion present and the defensive mechanisms of the organisms, i.e., the amount of leukocytes, etc.

I believe it is important to emphasize that the findings of the various investigators regarding the first appearance and the kind of cytological changes during radiation therapy are of value and comparable only when the kind of radiation technique applied is precisely specified because the radiation dosage and the duration of its action on the cell is decisive for the kind of visible effects on the cell morphology.

CLOSING REMARKS

OLLE KJELLGREN:

Glücksmann's observations are of great interest and they are in very good accordance with my findings. A priori one might expect that the changes observed in the tissue should precede by some time those observed in the exfoliated cells. If the vaginal radium dose (high dose ≥ 1700 mgh and low dose ≤ 1699 mgh) is taken into consideration I have, like Glücksmann, found that in the poor as well as in the good response cases a high radium dose is correlated with a delayed appearance of the radiation reaction.

I am of the same opinion as Boyes when he points out that the differences in results at various centres for radiation cytology might be dependent largely on different methods of radiotherapy, especially differences in fractionation.

The time, when the radiation reaction in the vaginal smear culminates after treatment, varies with different types of therapy, and I believe that each center has to do its own experiments in order to ascertain which is the optimal day after radium treatment for judging the intensity of the radiation reaction.

As Smolka points out, it is essential that the smear is obtained from the irradiated region in the top of the vagina. The importance of taking smears by correct technique is discussed in the topic entitled "Method of Prognosis after Irradiation by means of Exfoliative Cytology" in this Symposium.

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RESULTS OF RR CELL STUDIES

RUTH M. GRAHAM AND JOHN B. GRAHAM

Buffalo, New York, U.S.A.

In the first series of seventy-three cases in which the cytologic radiation response was used for prognosis, the five year survival rate was 55% for the good response group including all stages of disease and 8% for the poor response (1). The cases were rather evenly divided, there being thirty-six in the good response group and thirty-seven in the poor response group.

That there is a true permanency of healing of the malignancy is indicated by the ten year survival rate (2). The one case that survived five years in the poor response group survived ten years. All the patients who survived five years in the good response group survived ten years, except one. She had a recurrence in a cervical node at five years and nine months.

Another series of sixty-nine cases was studied in a prospective fashion after the original series. The five year survival rate of both the good and poor response group according to stage of disease in the combined series is given in Table I (3).

TABLE I

CANCER OF THE CERVIX TREATED RADIOLOGICALLY FIVE YEAR RESULTS				
Stage	Radiation reaction			
	Good RR		Poor RR	
	No.	Cured	No.	Cured
I	13	9	2	0
II	45	35	24	3
III	15	6	26	2
IV	4	0	13	0
	77	50 (65%)	65	5 (8%)

Since these two series, we have been trying to modify the response in the poor response patients so that we do not have more data on the poor response group except for a small group of 14 cases, all of whom died of their disease. However, it is of interest that the four year survival in the spontaneous good response group in the Stockholm series was similar to that of the original series -59%- even though the mode of treatment was quite different. In the Stockholm series, the patients were treated by radium primarily. In the series from the Massachusetts General Hospital, the primary treatment was by x-ray.

From these results it may be stated that the response of the benign cells is a critical factor in whether or not a patient will survive five years if treated by radiation for her carcinoma of the cervix. However, it should be pointed out that a good response to ionizing radiation in the benign cells does not guarantee survival by any means. The response of the normal cells is only one of several considerations such as the stage of the disease, the adequacy of the radiation therapy and general condition of the patient. All of these other factors are of real importance.

However, it can be stated that if the benign squamous cells do not show any response to radiation the possibility of surviving five years is not very good, even though the disease be limited in extent, the radiotherapy adequate and the condition of the patient good. By this cytologic prognostic method we can pick out a group of patients in whom other measures must be taken if the treatment is to be of value. Furthermore, we can recognize such patients at the time they are receiving their radiotherapy and we need not wait for a recurrence to know that the radiation has been ineffective in the control of the disease.

Bibliography

1. Graham, R.M.: Surg. Gyn. & Obst. 84:153, 1947.
2. Graham, R.M.: Acta Union International Contre le Cancer XIV:364, 1958.
3. Graham, R.M. and Graham, J.B.: Cancer 8:59, 1955.

OLLE KJELLGREN

Gothenburg, Sweden

Five-year apparent recovery rate. A series of 241 consecutive patients was used for analysis of the five year cure rate. Those patients were recorded as having made five year recoveries who, five years after commencement of therapy, were alive and presented no clinical signs of recurrence or metastatic spread. For the present purposes patients dying during an intercurrent disease were classified as having died of cancer.

Analysis of the five year recovery rates in the poor and good response groups ($36.5 \pm 4.3\%$ and $73.9 \pm 4.1\%$ respectively) shows that there was a highly significant preponderance among the patients with a good response ($\chi^2 = 33.907$, $df = 1$, $p < 0.001$) (Table I).

TABLE I

	Poor response	Good response	Total
Stage I	$63.9 \pm 8.0 \%$	$85.1 \pm 5.2 \%$	$75.0 \pm 4.7 \%$
Stage II	$32.8 \pm 6.0 \%$	$72.5 \pm 6.3 \%$	$58.9 \pm 4.6 \%$
Stage III	$12.0 \pm 6.5 \%$	$47.0 \pm 12.1 \%$	$26.2 \pm 6.8 \%$
Stage IV	0 %	—	0 %
Total	$36.5 \pm 4.3 \%$	$73.9 \pm 4.1 \%$	$54.4 \pm 2.8 \%$
Five year apparent recovery rates in Stages I to IV within the poor and good response groups.			

Broken down by international stages, the preponderance of the five year recovery rate in the group with good responses was found to be nearly significant for Stage I ($\chi^2 = 5.018$, $df = 1$, $0.05 > p > 0.02$), highly significant for Stage II ($\chi^2 = 17.572$, $df = 1$, $p < 0.001$) and nearly significant for Stage III ($\chi^2 = 4.748$, $df = 1$, $0.05 > p > 0.02$).

If poor response patients in Stage I are compared with good response patients in Stage II, the difference between the five year recovery rates of $63.9 \pm 8.0\%$ and $72.5 \pm 6.3\%$ respectively turns out to be not significant ($\chi^2 = 0.739$, $df = 1$, $0.50 > p > 0.30$) (Fig. 1).

The significance of endocrine factors for the cytological response has also been studied (Acta Radiol. suppl. 168, 1958).

Survival time. The length of time the patients remained alive after the commencement of therapy was studied. Figure 2 shows survival curves for the two response groups within the various clinical stages from the beginning of treatment up to five years thereafter. (The survivors in this case also include patients with signs of cancer.)

It was particularly interesting to observe the survival times of those 104 patients who died within the five year observation period. Survival times for both the poor and the good response groups among these patients are shown in Fig. 3. The good response patients dying after treatment tended to survive longer than the poor response patients who died. A year after the commencement of therapy the percentage of good response patients remaining alive was nearly significantly higher than the percentage of surviving poor response patients ($\chi^2 = 5.641$, $df = 1$, $0.02 > p > 0.01$). After 15 months, when 50% of the 104 patients had died, the deaths included $59.1 \pm 5.7\%$ of the poor response patients and merely $30.0 \pm 8.4\%$ of the good response patients, the difference being significant ($\chi^2 = 6.746$, $df = 1$, $0.01 > p > 0.001$). After 18 months the corresponding difference was nearly significant ($\chi^2 = 3.940$, $df = 1$, $0.05 > p > 0.02$).

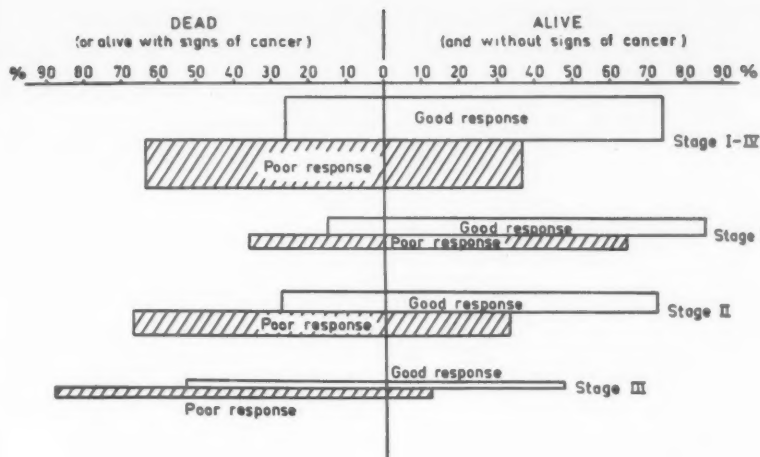


Fig. 1. Five year apparent recovery rates in the poor and good response groups.

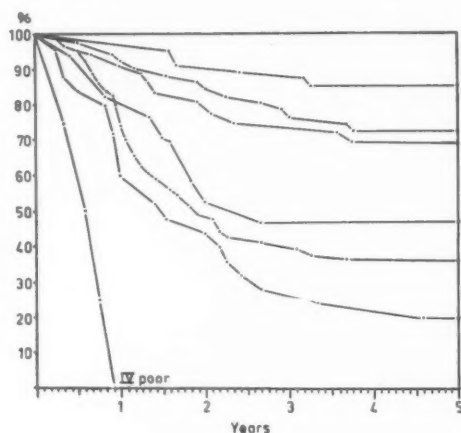


Fig. 2. Survival curves for the two response groups within various clinical stages.

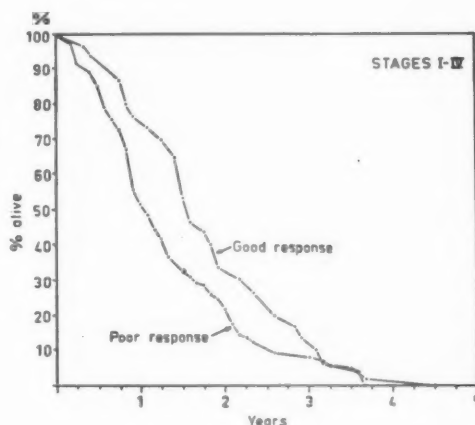


Fig. 3. Survival curves for 104 patients in the poor and good response groups dying within five years after the commencement of therapy.

Bibliography

1. Kjellgren, O.: Acta Radiol. suppl. 168, 1958.

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The material in this series consists of 145 cases of carcinoma of the cervix treated fully or in part at the Norwegian Radium Hospital. Examination of the slides was based on the criteria for changes in the normal cells as laid down by Ruth Graham, and the expression "good response" and "poor response" refers to the morphological picture of these cellular changes. Sixty per cent as a borderline between "good response" and "poor response."

The cases are histologically all squamous cell carcinomas.

All patients were first treated by radium application in one continuous dose, usually totaling 7200 mgh. Further treatment was either x-ray therapy, which followed immediately after the radium application (3000 r on each of 4 fields), or hysterectomy performed six to eight weeks after the radium treatment.

The results of this investigation as far as prognosis is concerned will be seen from Tables I and II.

TABLE I

	Good response		Poor response	
	No.	Cured	No.	Cured
Stage 0	8	8	4	4
Stage I	32	28	13	8
Stage II	30	29	40	10
Stage III	2	2	14	0
Stage IV	0	0	2	0
	72	67	73	22
		92%		30%

Prognosis for irradiated cancer of the cervix determined by exfoliative cytology. Follow-up: 5-1/2 years or more.

TABLE II

	Premenopausal				Postmenopausal			
	Good Resp.		Poor Resp.		Good Resp.		Poor Resp.	
	No.	Cured	No.	Cured	No.	Cured	No.	Cured
Stage 0	6	6	2	2	2	2	2	2
Stage I	26	22	10	7	6	6	3	1
Stage II	23	22	22	6	7	7	18	4
Stage III	1	1	4	0	1	1	10	0
Stage IV	0	0	0	0	0	0	2	0
	56	51	38	15	16	16	35	7
			40%				20%	

The 145 cases in Table I divided into premenopausal and postmenopausal groups.

The expression "cured" in the tables refers to the patient being alive and well without any sign of recurrence after a period of more than five years. The classification of the cases in Table II into premenopausal and postmenopausal groups is based on clinical information about the menopause, not on a fixed age.

As will be seen in Table I, there is a significant difference in the "cure" rate between the two groups "good response" and "poor response." From Table II one will see that there is further a marked difference in the cure rate in the "poor response" group before and after menopause, viz. 40% compared with 20%.

As shown in Table I, the present series contains 12 cases of cancer of the cervix, Stage 0. In four of these cases the smears showed the picture of "poor response" to the radium treatment. The number of cases in this group is too small to allow any conclusions, but the figures seem to indicate that for some reason or other the reaction of the normal epithelium to irradiation in early noninvasive carcinoma is less pronounced than in more advanced cases.

Persisting cancer cells in the smears after the entire radiation therapy was concluded has, in this series, proved to be a fatal sign. This occurred in 13 cases and all of these patients succumbed to their disease. The count of normal cells showing radiation changes in these 13 cases showed in all cases a "poor response," the percentage varying from 0 to 30.

As mentioned above, the radium treatment at the Norwegian Radium Hospital is given as one treatment, one continuous dose during 120 hours. This form of treatment will most probably influence the time of appearance of the cellular changes in the smears, and may account for the difference in the "cure" rate in the "poor response" group in this series as compared with the low figures in the series of Ruth Graham.

In the present series, the most frequent change observed in the normal cells after irradiation was vacuolization of the cytoplasm, and the second most frequent was nuclear changes. This may be explained by the difference in the method of radiation treatment at our hospital, and the time of taking the smears. Accordingly, this observation should confirm the claim of Ruth Graham that cellular size increase is a transient phenomenon which quite rapidly disappears.

As to the duration of cellular radiation changes in smears, I have not been able to find any general rule. Although the morphological changes in the normal cells might vanish during the first year after treatment was completed, one can also find cells showing evidence of irradiation treatment as long as 12 to 15 years afterwards. The difference in the observations of different investigators on this point may again be due to the various ways the irradiation treatment is carried out in different centers.

Closing this brief paper, I would like to point out that contrary to what one sees in cases of cancer of the breast, where recurrence often appears late (five years or more after treatment) recurrence in this series of cancer of the cervix occurred in the first one to three years after treatment, and the patients succumbed to their disease long before the end of five years.

In conclusion, I would say that this investigation confirms the observations of Ruth Graham and that the method she has established for determining prognosis after radiation treatment of cancer of the cervix should be a valuable means for selecting the right treatment of this disease in a great majority of cases.

Bibliography

1. Graham, R. M.: Transac. Sec. Ann. Meeting Inter-Soc. Cytology Council 2:63, 1954.
2. Graham, R. M.: Acta, Union Internat. contre le Cancer. 14:364, 1958.
3. Graham, R. M. and Goldie, K. R.: Cancer 1:71, 1955.
4. Graham, R. M. and Graham, J. B.: Cancer 8:59, 1955.
5. Kjellgren, Olle: Acta Radiol. Suppl. 168, 1958.
6. Messelt, Olaf. T.: Acta, Union Internat. contre le Cancer 14:367, 1958.
7. Nielsen, A. M.: Transac. First Internat. Cancer Cytology Congress 1:293, 1956.

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EXPERIMENTAL STUDIES ON THE CHEMICAL PROTECTION OF THE RECTUM OF RATS DURING PELVIC IRRADIATION

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I. INTRODUCTION:

Several chemical substances have been proven to be more or less effective radio-protectors: cyanide as well as cyanide-producing nitriles (2, 3, 6, 7, 15, 17, 18, 25), cystine (21, 22), glutathion (4, 8, 9, 16, 19, 20, 24), chelation factors (5), etc. Among them, cystamine and cysteamine are very satisfactory by the intensity of the radio-protection they confer and by their mild toxicity (12, 13, 23, 26). A review of the experiments performed to determine the efficacy of those different substances can be found in reference (1). As far as we know, those works have been achieved by administration of the radio-protector systemically. The possibility of a local protection conferred by a locally applied chemical substance has not been investigated, except by Forssberg (14), who proved that local use of cysteine protects the skin of guinea pigs against radiation.

Protection of the rectum against pelvic radiation could not be obtained by systemic administration of a chemical substance without simultaneous protection of the neighboring tissues, including the radiated tumors when present. Our goal in the following experiments has been to investigate the possibility of selective protection of the rectum against pelvic radiation by the use of cystamine.

II. MATERIAL, TECHNIQUE AND METHODS:

Material, radiation technique and cytologic methods are essentially the same as those outlined in our other report in this Symposium. However, in several of these experiments, the rectum has been chosen as the site of radiation rather than the vagina.

A daily smear was taken from the vagina and rectum of each animal.

Local administration of cystamine was provided by a continuous drip from a small flask connected to a very thin polyethylene tube. The distal end of the tube was introduced into the vagina or rectum, according to the experiment. The quantity so administered was either 5 cc of a 10% aqueous solution of 55 cc of a 1% aqueous solution. The drip was started 30 minutes prior to the onset of radiation and maintained during its entire duration.

III. RESULTS:

The results will be outlined in the next paragraphs. Details can be found elsewhere (10, 11).

A. The different aspects of radiation changes in vaginal smears, which have been described in our other report in this Symposium, were confirmed in the present series of experiments.

B. We consider as an irradiated rectal cell every cell which contains at least two nuclei, or the largest diameter of which is greater than 34 microns. The ratio of such cells is not over 7% in a normal rectal smear (Figs. 1-4).

C. In the rectum the evolution in time of radiation changes follows a course essentially similar to the one described in the vagina, although the latent period is shorter (one or two days).

D. After four hours of intrarectal radiation the average of highest percentages of irradiated cells is $26.9 \pm 1.6\%$ in the rectum and $24 \pm 1.7\%$ in the vagina.



Fig. 1.

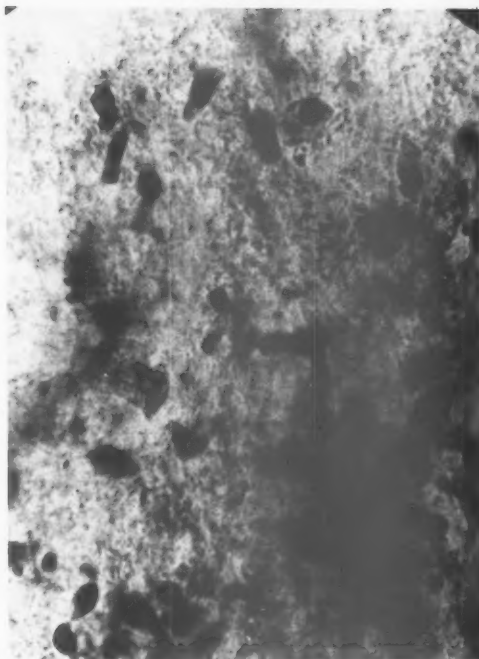


Fig. 2.

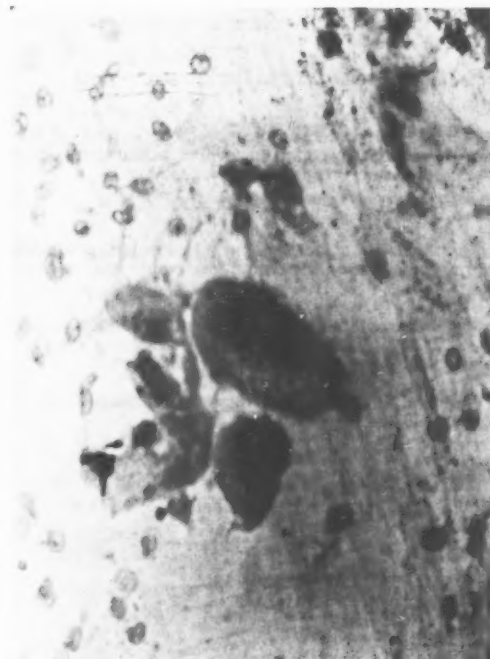


Fig. 3.

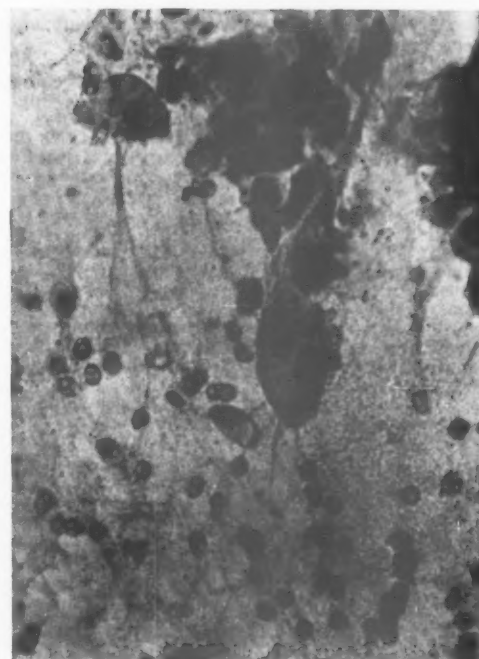


Fig. 4.

E. Our experiments on sytemic and local use of chemical radio-protection are summarized in Table I.

TABLE I
SYSTEMIC AND LOCAL PROTECTION OF RECTUM AND VAGINA
AGAINST LOCAL RADIATINN

RADIATION (RADIUM 50 mg x 4 hrs.)	PROTECTION	AVERAGE OF HIGHEST % OF RADIATED VAGINAL CELLS	AVERAGE OF HIGHEST % OF RECTAL CELLS (RADIATED)
INTRAVAGINAL	NONE	60.4 (\pm 3.4) (21 animals)	
	INTRAPERITONEAL (CYSTEAMINE 15 mg)	27 (\pm 2.8) (13 animals)	
	INTRAVAGINAL (CYSTAMINE 500 mg)	24.2 (\pm 1.3) (10 animals)	
INTRARECTAL	NONE	24 (\pm 1.7) (15 animals)	26.9 (\pm 1.6) (14 animals)
	INTRAPERITONEAL (CYSTEAMINE 15 mg)	17.6 (\pm 1.5) (12 animals)	19.2 (\pm 1.3) (11 animals)
	INTRARECTAL (CYSTAMINE 500 mg)	26.7 (\pm 1.8) (10 animals)	15.8 (\pm 1.2) (11 animals)

It can be seen that:

1. Systemic administration of cysteamine and intravaginal administration of cystamine protects the vaginal mucosa against intravaginal radiation.
2. Systemic administration of cysteamine protects the vaginal and rectal mucosa against intra-rectal radiation.
3. Intrarectal administration of cystamine protects the rectal mucosa but not the vaginal mucosa against rectal radiation.

We conclude from the experiment that the rectal mucosa is selectively protected; indeed, if its protection were due to partial resorption of cystamine into the general circulation (which would correspond to a systemic administration), the vagina also should be protected and this is not the case.

IV. COMMENT:

Selective radio-protection of the rectal mucosa by means of a locally applied chemical protector seems to be possible. As a matter of fact, similar experiments could be done on other easily accessible tissues like the skin, bladder, etc.

Postradiation rectal lesions represent an important problem in the treatment of cancer of the uterine cervix. Chemical protection of neighboring tissues, possibly combined to sensitization of the irradiated tumor, might lead to better therapeutic results and milder postradiation injuries.

Needless to say, more light should be shed on this problem by further experimental and clinical research before putting into practice the possible application that it implies.

Bibliography

1. Bacq, Z.M. and Alexander, P.: Principes de Radiobiologie-Sciences et Lettres. Liege, 1955.
2. Bacq, Z.M. and Herve, A.: Brit. J. Radio. 24:617, 1951.
3. Bacq, Z.M., Herve, A., Lecomte, J. and Fisher, P.: Science 111:356, 1951.
4. Bacq, Z.M. and Herve, A.: Bull. Acad. Med. Belg. 17:13, 1952.
5. Bacq, Z.M., Herve, A., and Fisher, P.: Bull. Acad. Med. Belg. 18:226, 1953.
6. Betz, H. and Herve, A.: C.R. Soc. Biol. 144:1015, 1950.
7. Bonet-Maury, P. and Patti, F.: J. Radiol. Electrol. 34:636, 1953.
8. Brues, A.M. and Patt, H.M.: Physiol. Rev. 33:85, 1953.
9. Chapman, W.H., Sipe, C.R., Elitzholtz, D.C., Cronkite, E.T. and Chambers, F.W.: Radiology 55:865, 1950.

10. Darcis, L. and Gilson, P.: *Experientia* 13:242, 1957.
11. Darcis, L. and Hotterbeex, P.: *Experientia* 14:18, 1958.
12. Deysson, G. and Truhaut, R.: *C.R. Acad. Sc. Paris* 236:2329, 1953.
13. Deysson, G. and Truhaut, R.: *Bull. Soc. Chim. Biol.* 35:1019, 1953.
14. Forssberg, A.: *Acta Radio. P.* 33:296, 1950.
15. Herve, A. and Bacq, Z. M.: *C.R. Soc. Biol.* 143:881 and 1158, 1949.
16. Hollaender, A. and Stapleton, G. E.: *Physiol. Rev.* 33:77, 1953.
17. Lorenz, W.: *Fortschritte Geb. - Roentgenstr. - Wiesbaden Congress*, 1952.
18. Mazzanti, L. and Franchi, M.: *Arch. Ital. Sc. Farmacol.* 2:261, 1952.
19. Ord, M. G. and Stocken, L. A.: *Physiol. Rev.* 33:356, 1953.
20. Patt, H. M.: *Physiol. Rev.* 33:35, 1953.
21. Patt, H. M., Smith, D. E., Tyree, E. B. and Straube, R. L.: *Proc. Soc. Exper. Biol. Med.* 73:18, 1950.
22. Patt, H. M., Tyree, E. B., Straube, R. L. and Smith, D. E.: *Science* 110:213, 1949.
23. Peczemik, O.: *Nature* 172:454, 1953.
24. Selle, W. A., Mason, G. D. and Newman, R. H.: *Univ. Calif. Atomic Proj. U. C. L. A.* 264, 1953.
25. Smith, D. E., Patt, H. M. and Tyree, E. B.: *Nucl. Sc. Abstr.* 5:2032, 1951.
26. Straube, R. L. and Patt, H. M.: *Proc. Soc. Exper. Biol. Med.* 84:702, 1953.

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THE NEXT SYMPOSIUM

VOLUME IV 1960 NUMBER 1

EFFECT OF ENDOGENOUS ESTROGENS ON THE VAGINAL EPITHELIUM (DISCUSSANTS ARE NOT LISTED)

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Comparative Study of Cytological Patterns and Steroid Hormone Excretion Values

MAX F. JAYLE, Ph. GENET, J. PUJOL and F. VEYRIN-FORRER, Paris, France

Histological Changes in the Epithelium and Connective Tissue of the Vagina and Ectocervix as a Result of Physiological Presence, Deficiency or Absence of Endogenous Estrogen Stimulation

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Cytological Changes of the Vaginal and Ectocervical Epithelium as a Result of Physiological Presence, Deficiency or Absence of Endogenous Estrogen Stimulation

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Cytological Changes of the Endocervical Epithelium as a Result of Physiological Presence, Deficiency or Absence of Endogenous Estrogen Stimulation

JEAN A. de BRUX, Paris, France and HENRIETTE WENNER-MANGEN, Luxembourg,
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Histo- and Cytochemistry of Basal and Parabasal Cells

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Histo- and Cytochemistry of Superficial Cells and Anucleated Squamous Cells

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Cytochemistry of Cytoplasmic Granules

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Terminology of Cytological Smears In Regard to Estrogen Effect

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Is the Karyopyknotic Index a Measurement of Endogenous Estrogens?

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Is the Presence of Epithelial Atrophy a Definite Criterion for Lack of Estrogen Production?

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The Value of Exfoliative Cytology in the Diagnosis of Follicular Persistency

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Vaginal Cytology in Hysterectomized Patients with Special Consideration to Presence or Absence of Ovarian Function After Hysterectomy

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The Value of Exfoliative Cytology in the Diagnosis of Hormone-Producing Tumors

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The Level of Endogenous Estrogens in Patients With Cervical Carcinoma as Demonstrated by Methods Other Than Exfoliative Cytology

ANDRÉ JULES BRET and FERNAND COUPEZ, Paris, France

The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Cervical Carcinoma

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The Level of Endogenous Estrogens in Patients With Ovarian or Endometrial Carcinoma as Demonstrated by Methods Other Than Exfoliative Cytology

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The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Untreated, Treated or Recurrent Breast Carcinoma (Excluding Those Cases Receiving Hormone Therapy or Who Have Undergone Extirpation of Endocrine Organs)

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The Cell Type (Normal Squamous Cells Only) in Vaginal Smears Of Patients With Breast Carcinoma After Ovariectomy (Those Treated by Sex Steroids and Those Without Additional Sex Steroid Administration Considered Separately)

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The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients With Breast Carcinoma After Adrenalectomy With or Without Additional Ovariectomy (Those Treated by Sex Steroids and Those Without Additional Sex Steroid Administration Considered Separately).

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The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients With Breast Carcinoma After Hypophysectomy With or Without Additional Adrenalectomy and/or Ovariectomy (Those Treated by Sex Steroids and Those Without Additional Sex Steroid Administration Considered Separately)

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Ovarian Function Following Pelvic Irradiation as Assessed by Vaginal Smears

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The Vaginal Smear During Menopause

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LUIS MONTALVO-RUIZ, Madrid, Spain
HANNAH PETERS, Copenhagen, Denmark
PETER STOLL, Heidelberg, Germany

FUTURE SYMPOSIA

The following Symposia by Correspondence are being prepared at this time or are planned for the future of ACTA CYTOLOGICA.

SYMPOSIUM BY CORRESPONDENCE ON VARIOUS TECHNIQUES OF OBTAINING MATERIAL FOR CYTOLOGICAL STUDIES (CLOSED)

SYMPOSIUM BY CORRESPONDENCE ON CARCINOMA IN SITU AND SO-CALLED PRECANCEROUS LESIONS (CLOSED)

SYMPOSIUM BY CORRESPONDENCE ON ENDOCERVICAL ADENOCARCINOMA (CLOSED)

SYMPOSIUM BY CORRESPONDENCE ON TRAINING OF CYTOTECHNOLOGISTS (CLOSED)

SYMPOSIUM BY CORRESPONDENCE ON THE EFFECTS OF PRE-GESTATIONAL AGENTS (ENDOGENOUS AND EXOGENOUS PREGESTATIONAL SUBSTANCES) (CLOSED)

SYMPOSIUM BY CORRESPONDENCE ON THE ORGANIZATION OF A LABORATORY OF EXFOLIATIVE CYTOLOGY

SYMPOSIUM BY CORRESPONDENCE ON CYTOLOGY OF THE RESPIRATORY TRACT

SYMPOSIUM BY CORRESPONDENCE ON SEX CHROMATIN

SYMPOSIUM BY CORRESPONDENCE ON GASTROINTESTINAL CYTOLOGY

SYMPOSIUM BY CORRESPONDENCE ON COMPARATIVE DIAGNOSTIC ACCURACY, EFFICIENCY AND SPECIFICITY OF TECHNIQUES FOR EARLY CANCER DETECTION.

SYMPOSIUM BY CORRESPONDENCE ON CYTOLOGY OF THE ASCITIC FLUID

SYMPOSIUM BY CORRESPONDENCE ON HISTIOCYTES

Individuals interested in participating in the above Symposia by Correspondence are invited to contact the Editorial Office for details. The six symposia listed on the top of this list are already closed; no new participants can be accepted. However, participants for the latter seven symposia are welcome, and may be listed upon request.

There will be no more listing of details concerning the FUTURE SYMPOSIA in the journal because space does not permit listing all participants, topics, and the various deadlines for each individual symposium. This information is readily available by writing to the Editorial Office, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A. Inquiries are invited.

SYMPOSIA UNDER CONSIDERATION

The following symposia have been suggested for consideration, but are not listed in the order of preference or chronology. The readers are invited to inform the Editorial Office in which of the symposia they would be most interested, so that an order of preference may be tentatively arranged. The following topics do not include the ones which are already scheduled, and therefore listed under FUTURE SYMPOSIA.

- Symposium on Tadpole-Shaped Squamoid Cells.
- Symposium on Cytological Studies in Amenorrhea.
- Symposium on Cytology of Malignant Tumors of Ovary and Tubes.
- Symposium on Extra-Genital Cytology of Metastatic Gynecological Lesions.
- Symposium on Phasemicroscopy and Other Special Microscopic Techniques.
- Symposium on Training of Exfoliative Cytologists.
- Symposium on Cytological Changes due to Microbiological Factors.
- Symposium on Cytological Microphotography.
- Symposium on Cytological Terminology for Hormonal Evaluation.
- Symposium on Quantitative Cytochemistry of Exfoliated Cells.
- Symposium on Vaginal Cytology During Childhood.
- Symposium on Cytology of Exudates.

ABSTRACTS

This portion of ACTA CYTOLOGICA includes abstracts (approximately 150-300 word each) of papers, either recently published or accepted for publication. Authors are invited to submit their own abstracts. Authors are requested to forward to the Editorial Office a complete manuscript or reprint of the original paper together with their abstract. All figures should be included.

The Editorial Office maintains a *free Literature Service* for distribution of available papers to cytologists. Authors are requested to send a minimum of 10 reprints, if possible, 150 copies of published papers to the Editorial Office. The Literature Service will make photostatic reproductions of papers which are unobtainable whenever possible.

RÉSUMÉS

Cette rubrique des ACTA CYTOLOGICA contient des résumés (d'environ 150 à 300 mots) de publications qui ont été récemment publiées ou acceptées pour la publication. Les auteurs sont priés de présenter leurs résumés *en anglais*. Les auteurs sont invités à faire parvenir au bureau de rédaction, en même temps que leur résumé, un manuscrit complet comprenant toutes les illustrations ou un tiré-à-part du travail original.

Le bureau de rédaction entretient un *service gratuit d'information littéraire* pour la distribution aux cytologistes de toute publication disponible. Les auteurs sont priés d'adresser au bureau de rédaction un minimum de 10, si possible 150 copies ou tirés-à-part de travaux publiés. Le service de documentation fera dans la mesure du possible des photocopies des publications épuisées.

ZUSAMMENFASSENDE BERICHTE AUS DER ZYTOLOGISCHEN LITERATUR

Dieser Teil der ACTA CYTOLOGICA beinhaltet zusammenfassende Berichte (von etwa 150 bis 300 Worten) von wissenschaftlichen Veröffentlichungen, die entweder schon publiziert oder zur Publikation angenommen worden sind. Autoren sind hiermit eingeladen, Zusammenfassungen ihrer Arbeiten (*in englischer Sprache*) an die Schriftleitung zu senden. Die Autoren sind gebeten, der Schriftleitung das vollständige Manuskript mit allen Abbildungen oder den Sonderdruck der Arbeit einzureichen.

Die Schriftleitung unterhält einen *kostenlosen Literatur-Dienst* zur Verteilung von wissenschaftlichen Arbeiten. Autoren sind gebeten, der Schriftleitung mindestens 10, möglichst aber 150 Kopien von Sonderdrucken ihrer Arbeiten einzureichen. Der Literatur-Dienst steht auch nach Möglichkeit zur Herstellung von Lichtkopien von schwer zugänglichen Arbeiten zur Verfügung.

RESUMENES

Esta parte de ACTA CYTOLOGICA incluye resúmenes (aproximadamente de 150-300 palabras cada uno) de los trabajos, publicados recientemente, o aceptados para su publicación. Los autores deberán enviar sus resúmenes *en inglés*. Se requiere a los autores para que envíen a la Oficina Editorial, junto con su resumen, un manuscrito completo o separata del trabajo original. Deberán incluirse todas las figuras.

La Oficina Editorial mantiene un *Servicio de Literatura, gratuito*, para la distribución de trabajos disponibles. Se ruega a los autores que envíen a la Oficina Editorial un mínimo de 10 copias de sus trabajos publicados y, de ser posible, 150 copias. El Servicio de Literatura hará, siempre que ello sea posible, reproducciones fotostáticas de los trabajos que los autores no puedan obtener.

CANCER CYTOLOGY

CYTOLOGY FOR UTERINE-CANCER DETECTION IN CLINIC AND PRIVATE PATIENTS

WALTER K. HARTFORD - Obst. & Gyn. 13:278, 1959.

Simultaneous studies of clinic patients in the University of Oklahoma Hospitals and private patients during the period 1948 through 1957. Incidence of detection of unsuspected carcinoma of the uteri reported as low as .9 per 1000 cases and as high as 5.7 per 1000 cases. In our experience with the University Hospital clinic patients, 71 unsuspected malignancies of the female genital tract were found among 17,671 patients examined. This is an incidence of 4.01 per 1000 patients examined. At the same time the private patient incidence was 42 among 11,415 women examined, and incidence of 3.6 per 1000. This is a total of 29,087 women examined with 113 unsuspected cancers found. The combined incidence is 3.9 per 1000.

Among the patients studied, both clinic and private, in one year, 1957, there were 133 biopsies reported because of Class III, IV or V, cytology reports. Cancer was not confirmed in 73 cases. It was confirmed in 60. The unconfirmed reports were found to be patients with varying degrees of atypicality such as, basal cell hyperplasia, epidermal hyperplasia, atypical hyperplasia and anaplasia.

In order to clarify the suspicious reports we have divided our Class III into a Class III Minus (-) and a Class III Plus (+) indicating to the clinician that in the "Minus group" we feel that it is atypical, not malignant, in the "Plus group" - an atypicality which is probably malignant. (Author's abstract)

CELLULAR CHANGES IN VAGINAL AND BUCCAL SMEARS AFTER RADIATION: AN INDEX OF THE RADIOCURABILITY OF CARCINOMA OF THE CERVIX

H. W. JONES, B. GOLDBERG, H. J. DAVIS and B. C. BURNS - Am. J. Obst. Gyn. 78:1083, 1959.

An effort was made to correlate the 2 year clinical end results with radiation changes in exfoliated vaginal cells according to the Graham technique. Unfortunately, in our hands, this was not successful.

The concept of generalized host radiosensitivity was examined by administration of a test dose of irradiation to the buccal mucous membrane after which certain changes in the exfoliated cells were observed. Two features of radiation cytotoxicity - macrocytosis and multinucleation - proved to lend themselves well to objective measurement and correlated in high degree with the clinical end results in the retrospective study of 50 Stage I and Stage II carcinomas of the cervix. With multinucleation as the sole criterion, the prognostic error was but 12 per cent in these operable patients. At the present time a prospective study is in progress. (Authors' abstract.)

THE RELATIONSHIP OF THE SQUAMOUS COLUMNAR JUNCTION AND THE ENDOCERVICAL GLANDS TO THE SITE OF ORIGIN OF CARCINOMA OF THE CERVIX

W. THORNTON, C. H. FOX and D. E. SMITH - Am. J. Obst. & Gyn. 78:1060, 1959.

The histologic findings in 200 cold-knife cone specimens of the uterine cervix have been presented. The variations in the anatomic location of the transformation zone have been noted, and these variations are shown in a series of schematic illustrations. In 28 specimens it was not possible to identify the squamo-columnar junction for reasons specified. The dysplastic and neoplastic lesions were found in the region of the transformation zone in all except 11 specimens of 172 in which it was possible to plot this zone. The epithelium of the endocervix or the endocervical glands was involved in 193 of the 200 specimens (96%). From these studies it would appear that the dysplastic and neoplastic processes, in all probability, began

in the basal cells lying beneath the columnar epithelium of the endocervical canal or the endocervical glands. This study would suggest that the squamous epithelium of the portio vaginalis is an infrequent site of origin of these changes.

These findings are of significant clinical importance in the early detection of neoplastic disease of the uterine cervix. Because of the variation in the anatomic location of the transformation zone, it would appear that exfoliative cytologic studies are the most satisfactory methods of detecting significant cellular changes in this zone. The high percentage of specimens in which it appears that the reserve cells are involved in these dysplastic and neoplastic changes would indicate that atypical exfoliative cytologic changes are best investigated by a tissue specimen which not only includes the transformation zone but also a significant portion of the endocervix for histologic study of the epithelium of the endocervix and endocervical glands. (Authors' abstract)

HORMONAL CYTOLOGY

THE METRORRHAGIA IN "MIXED ENDOMETRIUM" - HYPOPROGESTINIC ENDOMETRIUM WITH IRREGULAR RIPENING (LA METRORRAGIA IN "ENDOMETRIO MISTO" - L'ENDOMETRIO IPIPROGESTINICO CON MATURAZIONE IRREGOLARE)

UGO CITTI and GHERARDO CASAGLIA - Rivista Italiana di Ginecologia 42:36, 1959.

The authors report 25 cases of metrorrhagia in "mixed hypoprogesteric endometrium with irregular maturation" observed in 1250 patients with dysfunctional uterine bleeding, treated at the Department of Obstetrics and Gynecology of the University of Bologna in the three year period from 1955 to 1957.

Of the 25 cases clinically characterized by persistent hemorrhages starting from the 16th to 22nd day of the cycle, seven showed a simple glandular hyperplasia in addition to the secretory changes.

After brief review of the literature, the authors extensively describe four of the 25 cases from the clinical and histological point of view. The authors agree with the etiopathogenetic hypothesis of an absolute or relative functional defectiveness of the corpus luteum due to luteinizing hormone in the first phase of the cycle.

The authors compare the syndrome in question with the pseudomenstruation due to partial luteinization of unruptured follicles, and briefly describe the suggested therapy. (Authors' abstract)

INFLUENCE OF SEXUAL HORMONES ON THE VAGINAL SECRETION (SEXUAL HORMONERNE INDFLYDELSE PA VAGINAL SEKRETET)

POUL FLINDT-HANSEN - Thesis (with English summary) 194 pages, published Copenhagen, Denmark, December, 1957.

This thesis discusses the following points:

1. Hitherto used methods of demonstration of ovarian hormones and earlier investigations into cyclical changes of the vaginal mucosa and vaginal smears.
2. Preparation, staining and examination of vaginal smears with description of the different types of epithelial cells and description of the different types of epithelial cells and the various groups of smears: atrophic, hypotrophic and dystrophic.
3. Investigations of smears from 24 normal women (28 cycles) with special reference to cyclical, cytological variations throughout a normal cycle (Eosinophilic Index, folding-clumping, and leukocytic content).
4. Agreement between the cytohormonal examination and other investigations: endometrial biopsy, cervical secretion, basal temperature and hormone analyses.
5. Vaginal smears of hypo- and hyperestrogen types: new born female children, climacteric, post-climacteric, castrated and hysterectomized women and women with menstrual disorders (hypo-amenorrhea and meno-metrorrhagia).
6. Influence of estrogen and androgen treatment on the vaginal smear.
7. A few cases of genital cancer and cases with smears from the buccal mucosa. (Author's abstract)

THE ESTROGEN LEVEL IN WOMEN AS DETERMINED BY CYTOHORMONAL INVESTIGATIONS OF VAGINAL SMEARS

POUL FLINDT-HANSEN - Danish Medical Bulletin 6:122, June, 1959.

Twenty-eight normal Eosinophilic Index (E.I.) curves (based on the percentage content of red eosinophilic cells in the vaginal smear) are divided into three intermenstrual phases: postmenstrual, ovulative and premenstrual. Outer limits (scatter) were calculated for the Eosinophilic Index and duration of the phase in the three phases and normo estrogenism is defined as a periodic variation between various E.I. levels, which individually should not last for more than a couple of weeks. If the same values are present for longer periods than those stated, conditions of hyper- or hypoestrogenism are concerned. The limits for these are recorded with regard to the E.I. level and the duration of the phase.

Agreement between the cytohormonal investigation and excretion of estrogen in the urine was found in 90%. Endometrial biopsies in the proliferative phase showed agreement in 90%, while biopsies in the secretory and atrophic phase for different reasons could not be compared directly with the vaginal smear. (Author's abstract)

THE VAGINAL SMEAR IN AMENORRHEA AND ITS REACTION TO THERAPY

POUL FLINDT-HANSEN - Danish Medical Bulletin 6:126, June, 1959.

The cases of amenorrhea mentioned are subdivided into atrophic, hypotrophic and dystrophic cases. Treatment should consist mainly of stimulation therapy with gonadotrophic hormones. In approximately half of the cases bleeding ensued and, simultaneously, an increase of the Eosinophilic Index (E.I.) values occurred; conversely, cases without bleeding, by and large, did not show any increase of the E.I. curve.

The latent period for increase in the E.I. averaged five days.

Finally, the prognosis and the indications for treatment of amenorrhea are mentioned as judged from the cytohormonal types. (Author's abstract)

THE GLANDULAR STRUCTURES OF THE CERVIX UTERI DURING PREGNANCY

C. F. FLUHMANN - Am. J. Obst. & Gyn. 78:990, 1959.

This investigation was based on 39 specimens of the cervix uteri at different stages of gestation. They were studied by various methods including special photographic techniques, thick cleared sections, conventional microscopic preparations, serial microscopic sections, and reconstructions on sheets of transparent plastic material.

As in the nonpregnant state the cervical mucosa during pregnancy does not contain tubular racemose glands. The basic structure is a groove or cleft of varying dimensions which undergoes certain fundamental changes: (1) by a process of occlusion it forms tunnels which course more or less parallel to the surface and in the same general direction as the parent cleft; (2) the walls of the cleft may develop exophytic growths which project into the lumen and contribute to the formation of secondary clefts; (3) the secondary clefts themselves may form extrusions and tunnels; (4) as a result of enhanced growth and secretion during pregnancy these fundamental structures become greatly increased in size and number. The thickness of the cervical mucosa in term pregnancies measured from 3.0 to 6.0 mm as compared to 1.2 to 3.5 mm in the nonpregnant state. (Author's abstract)

COLPOCYTOLOGICAL FINDINGS IN PRE-MENSTRUAL SYNDROME (OSSERVAZIONI COLPOCITOLOGICHE SULLA SINDROME PREMENSTRUALE)

L. NOBILI and U. CITTI - Arch. Ost. Ginec. 62, 1957.

A series of 50 cases of premenstrual syndrome have been examined and investigated for a period of two to five months by means of cytologic studies. After a discussion on the symptomatology, the author is of the opinion that the syndrome has a hyper-folliculenic etiology (physical-hormonal syndrome). The remarkable results reported have been obtained by an association of methyl-testosterone, vitamin A and a diuretic compound such as neophyllina.

The few failures reported with such treatment are attributable to an overpowering psychical factor. The author, therefore, concludes that along with this treatment there should be added psychotherapy and sedative therapy. (Authors' abstract)

INDICATIONS AND CHOICE OF DOSAGE OF ANDROGENIC COMPLEMENTARY TREATMENT IN WOMEN SUFFERING FROM BREAST CANCER (LA PLACE DES FROTTIS VAGINAUX DANS LA CONDUITE DU TRAITEMENT HORMONAL COMPLEMENTAIRE DU SEIN)

A. SICARD and COLETTE MARSAN, - Presse Medicale, (accepted for publication).

Among the various complementary treatments of breast cancer, male hormonal therapy is thought to be one of the most active.

After a systematic study of the initial states of the vaginal mucosa in women suffering from breast cancer, and of the variability of the response of this mucosa to the different androgenic hormones, according to their nature and/or dosage, the authors proposed to fix the management of the treatment by vaginal cytological control.

The results, using 62 patients with follow-up examinations for several years, show that it is impossible to establish a standard therapeutic scheme and that treatment should be chosen according to individual properties. (Authors' abstract)

OTHER PHASES OF CYTOLOGY

TROPHOBLAST IN THE CIRCULATING BLOOD DURING PREGNANCY

G.W. DOUGLAS, L. THOMAS, M. CARR, N.M. CULLEN and R. MORRIS - Am. J. Obst. Gyn. 78:960, 1959.

1. Syncytiotrophoblast has been demonstrated in the circulating blood of pregnancy women at various stages, from the eighteenth week of gestation to term.
2. The migration of trophoblast into the blood stream appears to be a normal process in pregnancy, apart from complications and circumstances leading to maternal death.
3. The biologic implications of pregnancy as a form of homograft are discussed, and the possible importance of trophoblastic migration into the blood stream as a means of achieving tolerance to fetal tissues is suggested. (Authors' abstract)

THE SEX CHROMATIN IN HUMAN TUMORS (TUMORLERDE SEKS KROMATINININ DOKU FROTILERI ILE TETKIKI)

G. ESER - Tip Fak. Mec. (Istanbul) 22:679, 1959.

The sex characteristics of nuclei of 26 benign and 56 malignant tumors were studied. The method of sectioning is not always suitable for the study of the fine nuclear detail. Consequently, the procedure of tissue smear was used. Two hundred nuclei of tumor cells from each specimen prepared with Feulgen's stain were examined by means of oil immersion objective, for the presence of sex chromatin.

1. In benign tumors: This series consisted of 26 benign tumors, 19 were from females and 7 were from males. It is found that the nuclei of benign tumors and normal tissues in man are almost similar with respect to their morphological sex characteristic. The average incidences of sex chromatin are 66% in females and 7% in males.
2. In malignant tumors: This series consisted of 56 malignant tumors, 32 were from females and 24 were from males. The incidence of sex chromatin is lower in malignant tumors. The average incidence of a mass simulating the sex chromatin was 31.2% in females (even in some well-differentiated carcinomas) and 6.4% in males. We found that seven carcinomas of the breast (31.3%) in women had male type nuclei and three malignant tumors (two terato-carcinomas and one adeno-carcinoma of the stomach) in males had female type nuclei (51.5 - 40%). (Author's abstract)

THE VALUE OF THE EXAMINATION OF THE CEREBROSPINAL FLUID IN THE DIAGNOSIS OF INTRACRANIAL TUMORS

W.H. McMENEMEY and J.N. CUMINGS - J. Clin. Path. 12:400, 1959.

The cerebrospinal fluid findings are reviewed in 916 patients with histologically verified intracranial tumors, the cell count and protein values being analysed according to the different categories of tumor, and their significance discussed.

Pleocytosis is most commonly found in glioma, but on a percentage basis it is as common in carcinomatosis of the brain. Tumor cells, however, are rarely found in gliomas but frequently in carcinoma.

Thirteen cases are reported in which tumor cells have been identified in the cerebrospinal fluid. They include instances of pituitary adenoma (only once previously described) and, for the first time, of oligodendroglioma, chordoma, and meningioma.

A falling glucose level is highly suggestive of carcinomatous meningitis and the search for tumor cells should be intensified. A cell count within the limits of normal does not exclude this diagnosis. (Authors' abstract)

VAGINAL CYTOLOGY IN GLANDULAR CYSTIC HYPERPLASIA

MOJMIR SONEK - *Gynecologie et Obstetricque*. 58:96, 1959.

By systematic cytological investigation the author tried to explain the discord found in the vaginal cytological results in postmenopausal bleeding; especially concerning the estrogen in glandular cystic hyperplasia. Vaginal cytology was applied before, during and after the bleeding occurred. Also, the mucus crystallization test was employed. The examinations were performed regularly within short time intervals.

The author has classified his histological findings according to the criteria of Horalek; into functional and basal glandular cystic hyperplasias. This latter condition is considered an internal endometriosis. This classification provides precise histological criteria for both conditions. The cytological findings in functional glandular cystic hyperplasia, which represent 28% out of the total of 190 cases with postmenopausal bleeding, always reveal a high estrogenic activity. However, in basal glandular cystic hyperplasia (33% of the cases) the author found smears with low estrogenic activity of the mixed type.

The author proposes to use vaginal cytology as a tool for the differential diagnosis of the most frequent causes of postmenopausal bleedings.

ON THE GENESIS OF SO-CALLED INDIRECT METAPLASIA OF THE MULLERIAN DUCT SYSTEM (UBER DIE GENESE DER SOGENANTEN INDIREKTEN METAPLASIE IM BEREICH DES MULLER'SCHEN GANG-SYSTEMS)

WOLFGANG WALZ - *Z. Geburtsh* 151:1, 1958.

The process of indirect metaplasia can only be explained on the basis of the evolution of the genital system. By investigation of embryos of a crown of head to coccyx length of 44 mm up to a crown of head to foot length of 43 cm, it has been proven that the Mullerian duct system constitutes a genetical unit, not only in respect to its muscular-connective tissue part but even the total epithelial lining of the Fallopian tube, uterus and vagina is derived from the Mullerian epithelium. Thus, we arrive at the conclusion that each reserve cell within the Mullerian duct system has a plurivalent possibility of development because of common genesis, i. e., the reserve cells can be substituted not only for any particular epithelium, but they can form squamous epithelium in places where under normal conditions it cannot be found. This process has been called indirect metaplasia (Schridde). The conditions which cause formation of such a squamous epithelium are usually a change in the pH value of the particular locality, which in turn can be caused by congenital dislocations of epithelium, pregnancies, chronic inflammations and hormonal influences. These processes are investigated particularly within the juncture of endocervix and ectocervix, where the summation of these influences are maximum. (Author's abstract)

NEW BOOKS RECEIVED

AN INTRODUCTION TO GYNECOLOGICAL EXFOLIATIVE CYTOLOGY.

by WINIFRED LIU, 1959, Charles C. Thomas, Publisher, Springfield.

This monograph contains an introduction by Dr. Ruth M. Graham, and the chapters deal specifically with Anatomy, Histology, Endocrinology, Fundamentals of Cytology, Microscopy, General Principles of Microtechnique, Introduction to Exfoliative Cytology, Techniques of Exfoliative Cytology, Description of Cells, Description of Smears, Epithelial Cell Council in Normal Smears and Medical Terminology.

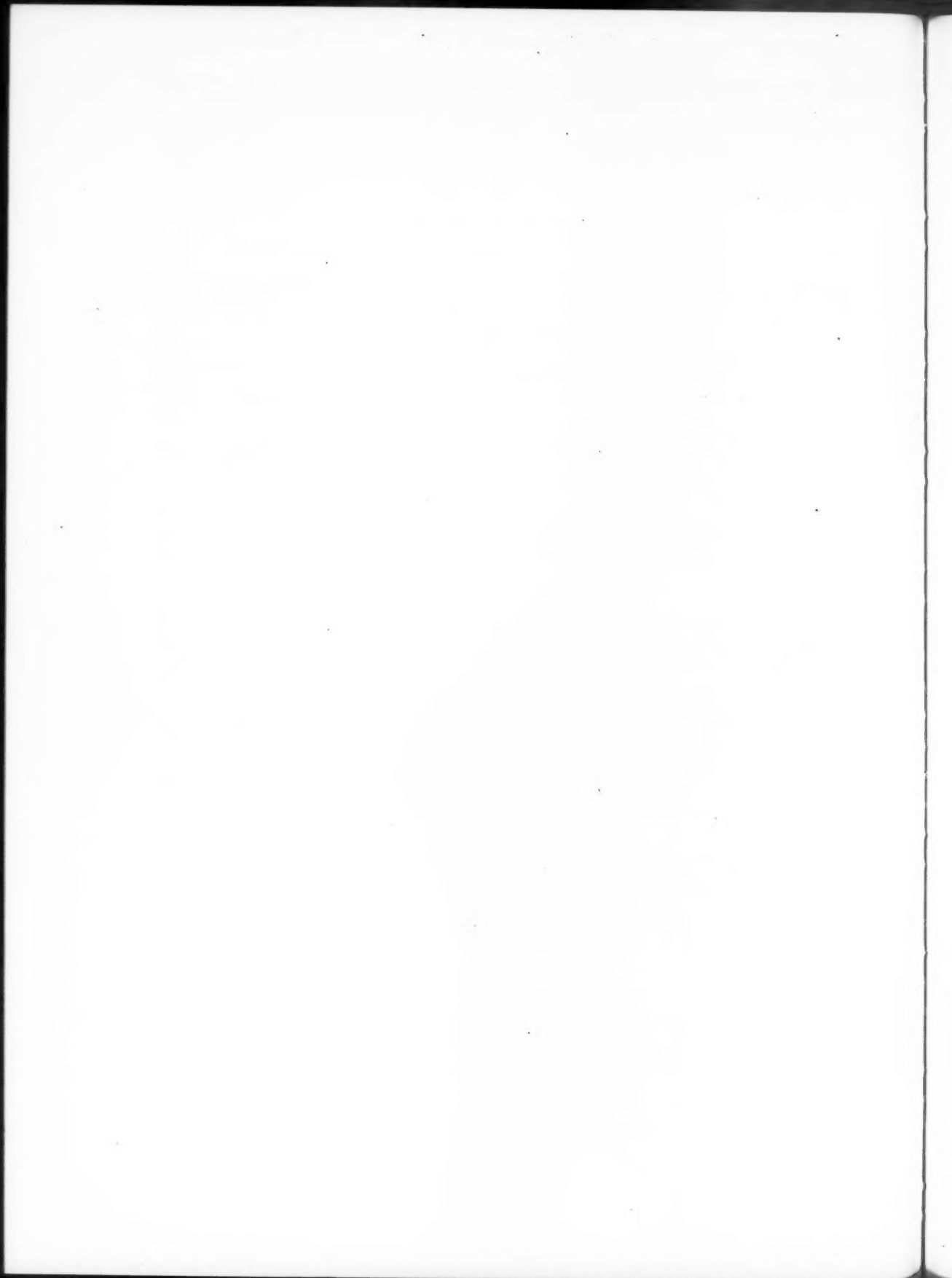
The book is well written and well illustrated as an introduction to the files of exfoliative cytology for the student in cytotechnology. In addition it is a book which fulfills a real need in the literature available for use in training laboratories and as a reference book.

COLPOSCOPIE.

by JULES BRET and FERNAND COUPEZ, 1960, Masson and Co., Publishers, Paris (in French).

This monograph opens with an introduction by Prof. Paul Funck-Brentano, and its chapters, which form a first section of the book, deal with the History of Colposcopy, Technique of Colposcopic Examination, Colposcopic Terminology, Colpophotography and Stereo-colpophotography. A second part of the monograph deals with individual colposcopic patterns as observed (1) on the normal ectocervix, (2) in inflammatory reactions, (3) in benign lesions, (4) in doubtful lesions, and (5) in colposcopically suspicious conditions. Another portion of the book is devoted to case reports and comparative statements on the efficiency of colposcopy and exfoliative cytology. These statements, however, contain no figures or statistical evaluation of the comparative diagnostic accuracy of the two techniques.

The book is well illustrated and also contains color photographs of colposcopic patterns. Published by Masson and Co., one of the leading publishers in gynecological and cytological literature in the French language, it provides an excellent introduction to the colposcopic technique for the French library. The authors are among the best of the colposcopists, and here they have presented a very worthwhile monograph for both the student and the advanced practitioner of the colposcopic examination of the ectocervix.



**THE INTERNATIONAL ACADEMY OF GYNECOLOGICAL CYTOLOGY
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BUSINESS MATTERS OF THE INTERNATIONAL ACADEMY

FROM THE OFFICE OF THE PRESIDENT

In accordance with the Bylaws of the International Academy Article I, Section 3, an

INTERNATIONAL CONGRESS OF EXFOLIATIVE CYTOLOGY

is scheduled for August 31 to September 2, 1961, immediately preceding the Third World Gynecology Congress. The place of the meeting will be Vienna, Austria.

Extracts from the Bylaws concerning the Scientific Session (Article 1, Section 10) : "... Papers presented at the Scientific Session shall be original papers which have never been presented or published. Material which has already been published or presented elsewhere may be considered in panel discussions. Fifty per cent of the Scientific Session shall be devoted to original papers, and fifty per cent of the time to panel discussions. . . ."

The attendance and participation is not restricted to members of the International Academy.

The Congress of Exfoliative Cytology will deal with, among other general topics, the following two main subjects:

- (1) Progression and regression of epithelial abnormalities, and
- (2) The hormonal activity as reflected in the vaginal smears in cases of gynecological tumors, breast tumors, and ovarian dystrophy.

In addition to other panel discussions, there will be an extensive discussion on cytological and histological terminologies and definitions.

Application forms for participants may be obtained from

The Office of the Secretary
International Congress of Exfoliative Cytology
666 Elm Street
Buffalo 3, New York, U.S.A.

ANNOUNCEMENT OF CYTOLOGICAL MEETINGS

1960: FIRST INTERNATIONAL CONGRESS ON HISTOCHEMISTRY AND CYTOCHEMISTRY, Paris, France, August 28 to September 3, 1960; President: Prof. J. Verne; Secretary General: Dr. R. Wegman, 54 rue Saints-Pères, Paris 6, France.

TENTH INTERNATIONAL CONGRESS FOR CELL BIOLOGY, Paris, France, September 4 to 9, 1960; President: Prof. J. Benoit; Secretary General: Prof. R.M. May, Faculté des Sciences, 12 rue Cuvier, Paris 5, France.

EIGHTH ANNUAL MEETING OF THE INTER-SOCIETY CYTOLOGY COUNCIL, Chicago, Illinois, U.S.A., September 23 to 25, 1960; President: A.E. Rakoff, M.D.; Secretary: Paul A. Younge, M.D., 1101 Beacon Street, Brookline 46, Massachusetts, U.S.A.

SECOND MEETING OF THE SOCIEDADE BRASILEIRA DE CITOLOGIA, Rio de Janeiro, Brasil, October 6 to 7, 1960; President: Doc. Dra. Clarice Amaral Ferreira; Secretary General: Dr. Edesio Neves, Moncorvo Filho 90, Caixa Postal 1289, Rio de Janeiro, Brasil.

1961: FIRST INTERNATIONAL CONGRESS OF EXFOLIATIVE CYTOLOGY, Vienna, Austria, August 31 to September 2, 1961; President: Prof. H.K. Zinser; Secretary General: Dr. Ruth M. Graham, 666 Elm Street, Buffalo 3, New York, U.S.A.

COURS SPECIAL DE CYTOLOGIE DESTINE AUX LABORANTINES

Un cours spécial de cytologie clinique destiné aux laborantines aura lieu, du Mercredi 8 juin au Jeudi 23 juin 1960, au Laboratoire de Colpocytologie du Professeur Agrégé Jean de Brux.

La première semaine sera consacrée aux techniques de prélèvement et aux diverses colorations, à la cytologie en rapport avec l'histophysiologie. La deuxième semaine sera plus spécialement consacrée au dépistage des lésions dysplasiques et carcinomateuses de l'appareil génital, broncho-pulmonaire et urinaire. De courtes leçons explicatives seront faites au début des travaux pratiques qui auront lieu tous les après-midi de 2 h 30 à 6 h.

Le nombre des participants est strictement restreint à 25.

Chaque demande d'admission doit être accompagnée d'un certificat du Chef de service indiquant les connaissances de la laborantine en microscopie.

Le prix de l'inscription est de 50 NF.

1ST INTERNATIONAL CONGRESS OF EXFOLIATIVE CYTOLOGY
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 in VIENNA, AUSTRIA

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INVITATION TO ATTEND AND PRELIMINARY INFORMATION

In the past two decades a new discipline has come to be of major importance in the diagnosis of early cancer—exfoliative cytology. It has been well established that cancer can be diagnosed earlier by means of exfoliative cytology than by any other means at present. Exfoliative cytology has also proved useful in studying the behavior of early lesions, and is becoming of increasing importance in the assessment of hormonal status.

To acquaint cytologists, clinicians, and pathologists with the new developments in this rapidly expanding field, an International Congress of Exfoliative Cytology is being held in Vienna, Austria, from August 31 to September 2, 1961. All interested in the field of exfoliative cytology are invited to attend.

Since the International Congress of Exfoliative Cytology will be immediately followed by the Third World Gynecology Congress, the main emphasis of this meeting will be on the exfoliative cytology of gynecology and obstetrics, though the exfoliative cytology of other specialties will not be excluded from the program.

The main topics of the Congress are:

1. Progression, Persistence, and Regression of Epithelial Anomalies (Reversibility and Irreversibility of Carcinoma in Situ)
2. Hormonal Activity as Reflected in the Cytological Specimen in Patients with Gynecologic Tumors or Breast Cancer.

In addition to these two themes of the Congress there will be general papers on various topics of exfoliative cytology and cytological research.

Simultaneous translation in four languages will be provided (English, French, German, and Spanish).

The complete transactions of the Congress will be published in ACTA CYTOLOGICA, the Journal of Exfoliative Cytology. Scientific medical exhibits are planned for the Congress.

The Congress Bureau has chartered an airliner from an international scheduled airline for persons (including their families) from the Americas planning to attend the Congress. This plane is scheduled to leave New York on August 19 (to Paris or Frankfurt) and scheduled to leave (for New York) on September 12. The price for the round trip fare is less than \$280.00.

IF YOU ARE INTERESTED IN ATTENDING OR PARTICIPATING IN THE INTERNATIONAL CONGRESS OF EXFOLIATIVE CYTOLOGY, AND/OR WISH TO HAVE MORE INFORMATION, PLEASE RETURN THE ATTACHED REPLY CARD AS SOON AS POSSIBLE.

USE THIS
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 AS SOON
 AS POSSIBLE



International Congress of Exfoliative Cytology
 666 Elm Street, Buffalo 3, New York, U.S.A.

Gentlemen:

I am interested in attending or participating in the International Congress in Vienna, Austria (Aug. 31–Sept. 2, 1961), and wish to have more information about the meeting. Please add my name to your mailing list:

Name: (Please print) _____

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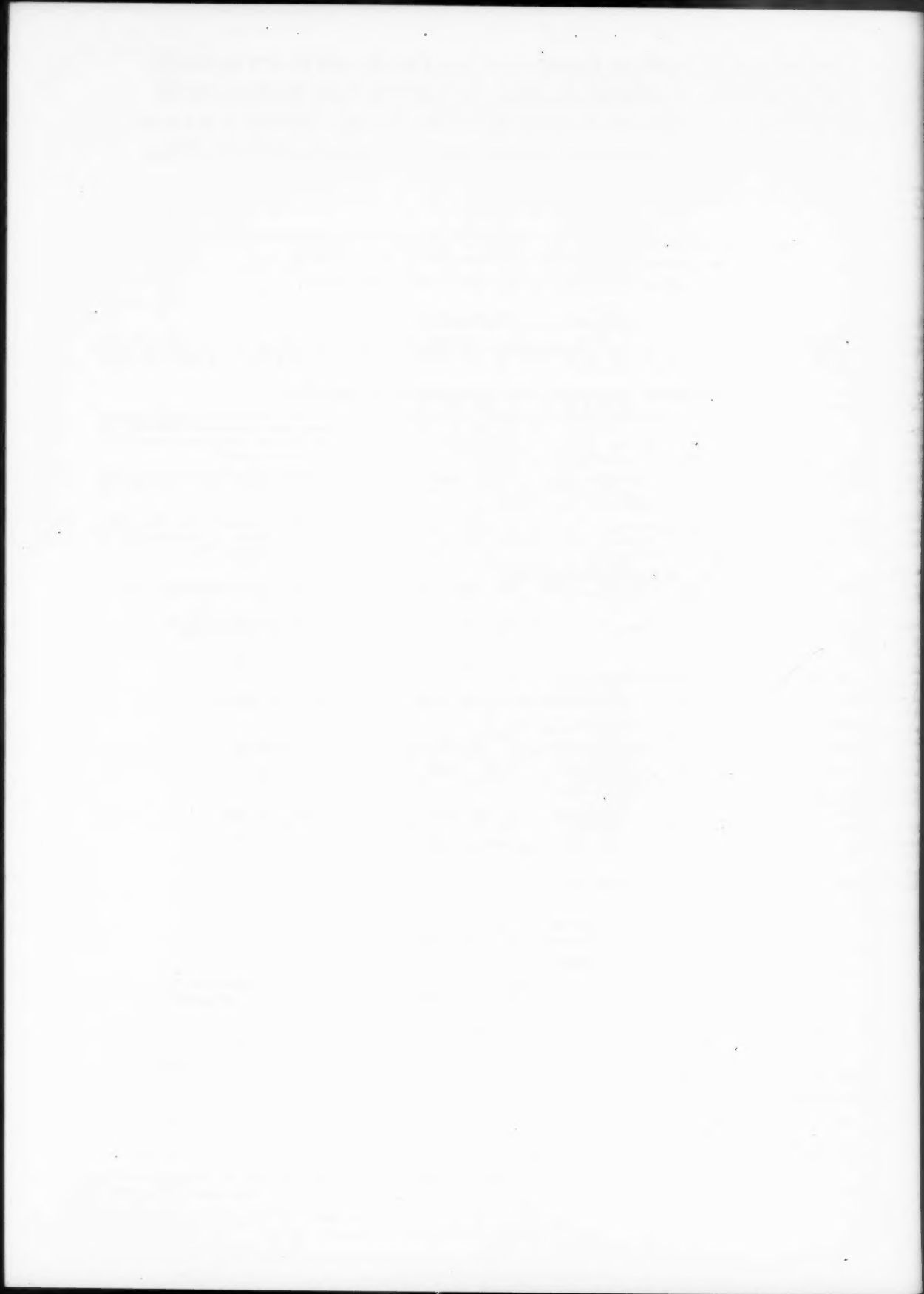
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I am interested in the reduced fare flight, New York–Paris (or Frankfurt)–New York
☐ Yes ☐ No (Check or circle answer which applies, please)

I am interested in attending the Third World Gynecology Congress (Sept. 3–10, 1961) after the International Congress of Exfoliative Cytology ☐ Yes ☐ No

I am interested in receiving information about subscription to ACTA CYTOLOGICA, the Journal of Exfoliative Cytology ☐ Yes ☐ No





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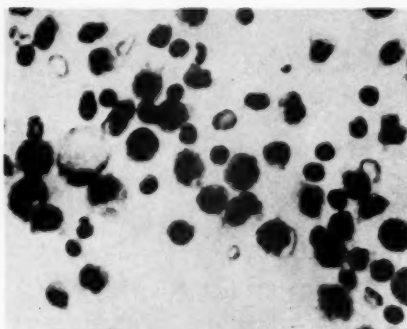
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CYTOLOGICALLY SPEAKING..

AN IMPORTANT USE of the Millipore Filter (MF®), and one which is of particular interest to the cytologist and cyto-technician, is in the area of exfoliative cytology of body fluids.

The MF® is a unique scientific tool that has revolutionized techniques in many fields — from microbiology to sterile filtration — from tissue culture to cytology. A thin cellulosic membrane, the MF® contains millions of capillary pores of uniform diameter per square centimeter of filter surface. Filters are available in eleven porosity grades ranging from 10 millimicrons (the approximate size of the polio virus) to 5 microns (the type used in the cytological procedure).

One of the most significant characteristics of the MF® is its absolute surface retention — on one plane — of all particles or cells larger than its pore size. The pores which pass through the MF® occupy 80 to 85% of the total filter volume. The unique characteristic permits extremely high flow rates for liquids and gases.



Exfoliative cells concentrated from urine sample on Millipore filter. (Photo courtesy Solomon et al., French Hospital, New York, N. Y.).

Cytologically speaking the MF® offers several advantages. A high degree of precision is obtained since all cells from a sample are quantitatively retained on the filter surface. For the analysis of most body fluids the clinical centrifugation procedure, which is quantitative only if all cells are of the same density, is eliminated. The amount of time saved is an important factor to be considered. Original morphological characteristics are not altered since at no time during the staining procedure are the cells allowed to dry — this coupled with the fact that the cells are located on one plane results in outstanding clarity and third dimensional appearance.



Typical apparatus used in the diagnostic laboratory.

The existing staining procedures such as the Papanicolaou procedure need be modified but slightly. We are recommending that as a final dehydrating step n-propanol be substituted for absolute ethanol in order to increase the rigidity of the filter. A mixture of absolute n-propanol and xylol in equal parts should be substituted for the ethanol-xylol mixture. Tests in several laboratories reveal that this modification allows excellent morphological and nuclear detail and that no malignant criteria are impaired.

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In a word — the Millipore technique has increased the accuracy of the diagnostic procedures involved in exfoliative cytology.

We would be glad to supply more information on Millipore Filters and procedures in this important field. Kindly address correspondence to Charles W. Fifield, Ph.D., Director of Biological Research.

1. Malmgren, R.A., Pruitt, J.C., DelVecchio, P.R., and Potter, J.F., 1958. A method for the cytologic detection of tumor cells in whole blood. *Jour. Nat'l Cancer Inst.*, 20: 1203-1213.

2. Solomon, C., Amelot, R.D., Hyman, R.M., Chaiban, R., Europa, D.L., 1958. Exfoliative cytology of the urinary tract: A new approach with reference to the isolation of cancer cells and the preparation of slides for study. *The Jour. of Urology*, 80: 374-382.

3. Seal, S.H., 1956. A method for concentrating cancer cells suspended in large quantities of fluid. *Cancer*, 9: 866-868.

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